Volume XXII November, 1926 Number 5

SOIL SCIENCE

Contents

PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
Baltimore, Maryland, U. S. A.

Made in United States of America

SOIL SCIENCE

FOUNDED BY

RUTGERS COLLEGE

NEW BRUNSWICK, N. J.

EDITORIAL BOARD

Entered as second-class matter May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879

INFLUENCE OF SULFUR AND GYPSUM ON THE SOLUBILITY OF POTASSIUM IN SOILS AND ON THE QUANTITY OF THIS ELEMENT REMOVED BY CERTAIN PLANTS[1]

O. M. SHEDD[2]

Kentucky Agricultural Experiment Station

Received for publication March 10, 1926

HISTORICAL

Ames and Boltz (1) have shown that sulfur, alone or in connection with other treatments, increased the water-soluble potassium in some Ohio soils, but they attribute the benefit to salt action rather than to acidity generated by the oxidation of the sulfur. Later, Ames and Simon (3) found that gypsum also appreciably increased the water-solubility of potassium in some soils.

André (4) reports increased solubility of potassium in microcline in contact with gypsum or calcium carbonate.

Bradley (6) carried on experiments on some Oregon soils by permitting the moist samples to remain in contact with lime or gypsum for several weeks in some instances. His conclusions were that gypsum acts as an indirect potassium fertilizer but that lime does not, in fact, lime sometimes depressed the solubility of potassium.

Briggs and Breazeale (7) conclude that availability to plants of the potassium in soils derived from orthoclase-bearing rocks is not increased by the addition of lime or gypsum; in fact, gypsum sometimes depresses potassium solubility. Their conclusions are based both on solubility studies and on the potassium content of wheat seedlings grown in the solutions.

Burgess (9) showed that gypsum increased the water-soluble potassium in a California soil which was unproductive but it had no effect on plant growth.

Dumont (11) found that gypsum increases the water-soluble potassium in some granitic soils.

Fraps (12) concludes from his solubility studies on Texas soils that lime or gypsum has very little, if any, effect on rendering potassium available to plants.

Lipman and Gericke (16) conducted experiments on three California soils with lime and gypsum. They obtained increased solubilities of potassium in water with both materials in two but not in the third soil, which was of a different type. They conclude that the apparently contradictory results found by different investigators can be attributed to the use of different types of soil in the experiments.

McCall and Smith (19) studied the effect of composting samples of Maryland and New Jersey greensands with sulfur, manure, and soil. They obtained large amounts of water-soluble potassium in some mixtures when sulfur was present. Their results indicate that their procedure may prove to be a practical and efficient method of obtaining available potassium from comparatively insoluble materials.

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[1] Published by permission of the Director of the Kentucky Agricultural Experiment Station.

[2] The author desires to express his thanks to Dr. A. M. Peter, Head of the Department of Chemistry, for helpful criticisms during this investigation and in the preparation of the manuscript.

McMiller (20) found that when some Minnesota soils were mixed with gypsum and then kept in a moist condition for some time, marked increases in potassium soluble in water sometimes were obtained. He attributes the negative results found by some investigators to unnatural conditions of contact of soil and gypsum.

Morse and Curry (21) report that lime and gypsum in contact with feldspar give increased water-soluble potassium but their effect is negligible in mixtures of feldspar and clay. They conclude that any solvent action which these materials might have on potassium of feldspar in a soil would be counteracted by the adsorptive property of its clay for this constituent.

Rudolfs (22) in his experiments on composting New Jersey greensand and sulfur found that small amounts of water-soluble potassium were liberated.

The foregoing references are conflicting regarding the positive action of gypsum in liberating potassium from different types of soil. Apparently, gypsum may liberate potassium in some types of soil but not in others. In view of recent experiments, the assertions made by some of the earlier writers, such as Hilgard (14, p. 379) and Storer (24, p. 207), that this material liberates potassium from soil silicates and supplies this essential ingredient in a soluble form is seen to be too general. Probably it would be more accurate to state, as mentioned by Lyon and Buckman (18, p. 379), that although gypsum has generally been credited with having the property of liberating potassium in soils, the experimental evidence is conflicting.

OBJECT OF THIS INVESTIGATION

The writer has been studying, for some time, the effect of S in Kentucky soils and has shown that it is readily oxidized by the sulfofying organisms of the soil to H_2SO_4. This acid then reacts with the phosphate in the soil or with $Ca_3(PO_4)_2$ which may have been added, to form soluble P compounds (23). This fact was first announced by Lipman and co-workers (17) and was later confirmed by other investigators (2, 8).

As the work regarding the effect of S on the liberation of K in soils is meager and that concerning the action of $CaSO_4 \cdot 2H_2O$ is conflicting, it was thought that further studies of this character on several types of Kentucky soils might contribute something of interest to this subject.

PLAN OF THE EXPERIMENTS

A large majority of the experiments referred to above involved additions of rather large amounts of either S or $CaSO_4 \cdot 2H_2O$ to the soil. The quantities varied from 250 to over 300,000 p.p.m. of soil and, with few exceptions, they were larger than correspond to field applications. As such large amounts are abnormal, it was decided for these experiments to employ quantities that do not greatly exceed those used in practice.

The soils selected were of different types found in Kentucky and representative of large areas. They were as follows:

Graves County. Untreated plots of experiment field. Yellowish-brown silt loam, undulating phase.

Fayette County. Untreated plots of experiment field. Light brown silt loam corresponding to Hagerstown silt loam—United States Bureau of Soils.

McCracken County. Untreated plots of experiment field. Yellowish-brown silt loam.

Taylor County. Untreated plots of experiment field. Yellowish-brown silt loam.

Shelby County. Composite of two soils from Eden formation. Light yellow silt loam.

Franklin County. Composite of three soils from Eden formation. Medium yellowish-brown silty clay loam.

Madison County. No. 1—Composite of two soils from Eden formation. (Hagerstown stony clay— United States Bureau of Soils.) Brown clay loam.

Madison County. No. 2—Untreated plots of experiment field. (Dekalb silt loam—United States Bureau of Soils) Yellowish-gray silt loam to silty clay loam.

Muhlenburg County. Untreated plots of experiment field. (Tilsit silt loam—United States Bureau of Soils) Yellowish-brown silt loam.

Logan County. Untreated plots of experiment field. (Decatur silt loam—United States Bureau of Soils) Reddish-brown silt loam.

Laurel County. Untreated plots of experiment field. Yellowish-brown silt loam to silty clay loam.

The samples were air-dried and put through a 2-mm. sieve. Nothing was added other than the chemicals mentioned in the tables. The required amount of each chemical was intimately mixed with the dry soil after which distilled H_2O was added to the extent of 20 per cent of the weight of air-dried soil. The moist samples were in good physical condition and could be stirred without packing.

The experiments were carried on in pint glass jars which had been previously weighed and were always kept covered with watch glasses. At intervals of every two or three weeks, the necessary quantity of distilled H_2O was added to replace the small amount lost by evaporation. Each time this was done the soil was thoroughly stirred.

The quantities of S or $CaSO_4 \cdot 2H_2O$ added were 250 p.p.m. of air-dried soil unless otherwise noted in the tables. The amount of $CaCO_3$, where applied, was 4000 p.p.m. The chemicals were the best C.P. grade and were previously tested to insure the absence of impurities which would affect the results.

The soils were kept in the jars, at laboratory temperature, for 4 months, at the end of which time each sample was stirred, after adding the required quantity of distilled H_2O to restore its initial moisture content, and portions were weighed out for the digestions, all of which except those mentioned later were made on the moist soil.

The determinations were total K and SO_4 on the original air-dried soils and soluble K, total SO_4, and hydrogen-ion concentration on the treated soils. The last two determinations were made on the air-dried samples and the soluble K was made on the moist soils except those with $0.2N$ HNO_3 described later.

The solubility of the K was determined by digestions in distilled H_2O, in $0.2N$ HNO_3, and in either $0.1M$ or $0.2M$ NH_4NO_3.

The $0.2N$ HNO_3 digestions were made on the air-dried soil remaining after extracting with distilled H_2O. The digestions in NH_4NO_3 solution were for comparison with those by HNO_3 so as to determine what effect the equivalent concentration of the neutral salt solution of that acid would have on the soil.

The amounts of H_2O and $CaCO_3$ present in the soils were taken into consideration in preparing the solvents for the various digestions so as not to affect their volume and strength.

In addition to the above, analyses were made of the content of K in young wheat and buckwheat plants grown on the soils after treatment. These will be described later.

TABLE 1

Graves County soil—Total K, 14,200 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	*per cent**	*gm.*	*per cent†*	*gm.*	
None	Distilled H_2O	19	0.13	0.0091	2.59	0.35	Wheat
	0.2*N* HNO_3	69	0.49				
	Sum	88	0.62				
	0.1*M* NH_4NO_3	151	1.06				
S	Distilled H_2O	22	0.15	0.0058	1.88	0.31	
	0.2*N* HNO_3	66	0.46				
	Sum	88	0.61				
	0.1*M* NH_4NO_3	305	2.15				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	19	0.13	0.0062	2.08	0.30	
	0.2*N* HNO_3	65	0.46				
	Sum	84	0.59				
	0.1*M* NH_4NO_3	250	1.76				
$CaCO_3$	Distilled H_2O	12	0.08	0.0162	1.46	1.11	Buckwheat
	0.2*N* HNO_3	65	0.46				
	Sum	77	0.54				
	0.1*M* NH_4NO_3	56	0.39				
S and $CaCO_3$	Distilled H_2O	12	0.08	0.0157	1.45	1.08	
	0.2*N* HNO_3	66	0.46				
	Sum	78	0.54				
	0.1*M* NH_4NO_3	59	0.42				
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	Distilled H_2O	11	0.08	0.0125	1.30	0.96	
	0.2*N* HNO_3	63	0.44				
	Sum	74	0.52				
	0.1 *M* NH_4NO_3	58	0.41				

* Of the total K.
† In air-dry plants.

METHODS OF ANALYSIS

Total K. Modified J. L. Smith method (5). In washing the fusion residue, only about 300 cc. of hot distilled H_2O was used which removed the K. The K_2PtCl_6 was treated with acid C_2H_5OH (10 volumes C_2H_5OH, 95 per cent: 1 conc. HCl) and allowed to stand over night after which the K_2PtCl_6 was

filtered and washed with acid C_2H_5OH, then with the usual NH_4Cl solution, and finally with C_2H_5OH (90 per cent).

H_2O-soluble K. To 132 gm. of the moist soil (110 gm. air-dried) 1078 cc. of distilled H_2O was added and digested for 4 days at room temperature, with shaking at 3-hour intervals each day. The whole was then transferred to a folded filter and the solution refiltered through the soil until clear. A liter of the filtrate, equal to 100 gm. of air-dried soil, was evaporated to a small volume, 5 cc. of alumina cream added to precipitate any trace of soil, filtered and washed. The filtrate was evaporated to dryness after adding 1 cc. H_2SO_4 (1:1). The residue was carefully ignited at low red heat to eliminate organic matter and the residue digested in HCl (1:1) for 2 hours on the steam bath.

TABLE 2

Fayette County soil—Total K, 14,000 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	*per cent**	*gm.*	*per cent†*	*gm.*	
None	Distilled H_2O	23	0.16	0.0044	1.92	0.23	Wheat
	$0.2N\ HNO_3$	107	0.76				
	Sum	130	0.92				
	$0.1M\ NH_4NO_3$	152	1.09				
S	Distilled H_2O	29	0.21	0.0076	2.93	0.26	
	$0.2N\ HNO_3$	103	0.73				
	Sum	132	0.94				
	$0.1M\ NH_4NO_3$	136	0.97				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	21	0.15	0.0040	1.92	0.21	
	$0.2N\ HNO_3$	109	0.78				
	Sum	130	0.93				
	$0.1M\ NH_4NO_3$	107	0.76				

* Of the total K.

† In air-dry plants.

It was filtered, H_2PtCl_6 added to the filtrate, the latter evaporated and K determined by treatment with acid C_2H_5OH as described under total K.

0.2 normal HNO_3-soluble K. The soil residue on the filter, obtained in the distilled H_2O digestion, was allowed to air dry. It was returned to the same bottle and 1100 cc. $0.2N\ HNO_3$ added. The soil was digested at room temperature for 5 hours, being shaken at 30-minute intervals, and the solution was then filtered until clear as previously described. An aliquot equivalent to 100 gm. air-dried soil was then concentrated to a small volume and 5 cc. conc. HNO_3 added. This was evaporated to dryness, treatment with HNO_3 was repeated to eliminate organic matter, and finally it was evaporated twice with 5 cc. conc. HCl. The residue was dried at 120°C. for several hours to dehydrate SiO_2, HCl (1:1) was added and the whole digested on the bath, filtered, and K determined in the same manner previously described.

NH_4NO_3-soluble K. The digestion was made on the same amount of moist soil in the manner described for the distilled H_2O extraction. The filtration was made as previously described and an aliquot of the filtrate equivalent to 100 gm. air-dried soil was evaporated to dryness. The bulk of NH_4 salt was eliminated at low heat, 1 cc. H_2SO_4 (1:1) was then added and the residue

TABLE 3

McCracken County soil—Total K, 16,200 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	*per cent**	*gm.*	*per cent†*	*gm.*	
None	Distilled H_2O	30	0.19	0.0091	2.93	0.31	Wheat
	0.2*N* HNO_3	90	0.55				
	Sum	120	0.74				
	0.1*M* NH_4NO_3	130	0.80				
S	Distilled H_2O	45	0.28	0.0087	2.80	0.31	
	0.2*N* HNO_3	81	0.50				
	Sum	126	0.78				
	0.1*M* NH_4NO_3	243	1.50				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	31	0.19	0.0080	3.20	0.25	
	0.2*N* HNO_3	92	0.57				
	Sum	123	0.76				
	0.1*M* NH_4NO_3	178	1.10				
$CaCO_3$	Distilled H_2O	21	0.13	0.0142	1.77	0.80	Buck-wheat
	0.2 *N* HNO_3	106	0.65				
	Sum	127	0.78				
	0.1*M* NH_4NO_3	93	0.57				
S and $CaCO_3$	Distilled H_2O	25	0.15	0.0197	1.89	1.04	
	0.2*N* HNO_3	107	0.66				
	Sum	132	0.81				
	0.1*M* NH_4NO_3	95	0.59				
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	Distilled H_2O	22	0.14	0.0091	1.06	0.86	
	0.2*N* HNO_3	102	0.63				
	Sum	124	0.77				
	0.1*M* NH_4NO_3	124	0.77				

* Of the total K.

† In air-dry plants.

ignited at low red heat to get rid of all NH_4 salt. The residue was taken up with HCl (1:1), digested on a steam bath, filtered, and K determined in the usual manner.

Sulfates. Ten grams air-dried soil was shaken with 200 cc. HCl (1 per cent) for 7 hours in a shaking machine, after which the solution was filtered until

clear. An aliquot was concentrated, 2 cc. HCl (1:1) and an excess of hot $BaCl_2$ solution (10 per cent) were slowly added. After being heated and standing over night, the $BaSO_4$ was filtered, washed, and determined in the usual manner. The precipitate after being weighed was treated with concentrated

TABLE 4

Taylor County soil—Total K, 6,500 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	*per cent**	*gm.*	*per cent*†	*gm.*	
None	Distilled H_2O	47	0.72	0.0118	3.57	0.33	Wheat
	$0.2N$ HNO_3	104	1.60				
	Sum	151	2.32				
	$0.1M$ NH_4NO_3	162	2.49				
S	Distilled H_2O	64	0.98	0.0090	2.66	0.34	
	$0.2N$ HNO_3	96	1.48				
	Sum	160	2.46				
	$0.1M$ NH_4NO_3	252	3.88				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	48	0.74	0.0048	2.18	0.22	
	$0.2N$ HNO_3	106	1.63				
	Sum	154	2.37				
	$0.1M$ NH_4NO_3	109	1.68				
$CaCO_3$	Distilled H_2O	18	0.28	0.0161	1.55	1.04	Buckwheat
	$0.2N$ HNO_3	96	1.48				
	Sum	114	1.76				
	$0.1M$ NH_4NO_3	111	1.71				
S‡ and $CaCO_3$	Distilled H_2O	23	0.35	0.0147	1.50	0.98	
	$0.2N$ HNO_3	97	1.49				
	Sum	120	1.84				
	$0.1M$ NH_4NO_3	121	1.86				
$CaSO_4 \cdot 2H_2O$‡ and $CaCO_3$	Distilled H_2O	16	0.25	0.0115	1.22	0.94	
	$0.2N$ HNO_3	102	1.57				
	Sum	118	1.82				
	$0.1M$ NH_4NO_3	104	1.60				

* Of the total K.

† In air-dry plants.

‡ The amount of S or of $CaSO_4 \cdot 2H_2O$ added was 500 p.p.m. instead of the usual application of 250 p.p.m.

H_2SO_4 and HF to eliminate traces of SiO_2, and was again weighed. No impurity that would have influenced the result was found to be present.

Hydrogen-ion concentration. A colorimetric method was used and the indicators were those recommended by Clark and Lubs (10, p. 66). The procedure

TABLE 5

Shelby County soil—Total K, 32,600 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	*per cent**	*gm.*	*per cent*†	*gm.*	
None	Distilled H_2O	19	0.06	0.0074	2.38	0.31	Wheat
	0.2*N* HNO_3	136	0.42				
	Sum	155	0.48				
	0.1*M* NH_4NO_3	217	0.67				
S	Distilled H_2O	28	0.09	0.0048	2.08	0.23	
	0.2*N* HNO_3	134	0.41				
	Sum	162	0 50				
	0.1*M* NH_4NO_3	226	0.69				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	20	0.06	0.0069	2.31	0.30	
	0.2*N* HNO_3	133	0.41				
	Sum	153	0.47				
	0.1*M* NH_4NO_3	188	0.58				

* Of the total K.

† In air-dry plants.

TABLE 6

Franklin County soil—Total K, 35,100 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	*per cent**	*gm.*	*per cent*†	*gm.*	
None	Distilled H_2O	19	0.05	0.0058	2.15	0.27	Wheat
	0.2*N* HNO_3	161	0.46				
	Sum	180	0.51				
	0.1*M* NH_4NO_3	192	0.55				
S	Distilled H_2O	24	0.07	0.0100	2.87	0.35	
	0.2*N* HNO_3	159	0.45				
	Sum	183	0.52				
	0.1*M* NH_4NO_3	203	0.58				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	18	0.05	0.0104	2.67	0.39	
	0.2*N* HNO_3	148	0.42				
	Sum	166	0.47				
	0.1*M* NH_4NO_3	185	0.53				

* Of the total K.

† In air-dry plants.

was to take 0.2 gm. of air-dried soil previously ground in a mortar and moisten it on an unglazed porcelain plate with 4 or 5 drops of aqueous indicator, sufficient being added so that the soil was saturated and the liquid had a tend-

ency to leave the soil when the plate was inclined. After 1 minute the liquid was drawn away from the soil with the point of a knife, spread, and the color compared with the Clark and Lubs standard color chart (10, p. 41). This method probably would be expected to give only comparative results but no difficulty was found in duplicating with different indicators where they could be used. Moreover it shows large differences in the same soil, with and without the addition of $CaCO_3$, and for this purpose mainly it was used.

K in plant ash. LeClerc and Breazeale (15) have shown that young wheat seedlings for a few weeks after sprouting have a great avidity for extracting K from culture solutions. It was thought that possibly, because of occasion by the soil, the K liberated by the treatments might not be extracted by the solvent and yet might be utilized by these plants. Therefore, their procedure

TABLE 7

Madison County soil No. 1—Total K, 29,100 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	*per cent**	*gm.*	*per cent*†	*gm.*	
None	Distilled H_2O	21	0.07	0.0067	2.30	0.29	Wheat
	0.2*N* HNO_3	195	0.67				
	Sum	216	0.74				
	0.1*M* NH_4NO_3	380	1.51				
S	Distilled H_2O	24	0.08	0.0161	3.84	0.42	
	0.2*N* HNO_3	186	0.64				
	Sum	210	0.72				
	0.1*M* NH_4NO_3	240	0.82				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	33	0.11	0.0086	2.68	0.32	
	0.2*N* HNO_3	176	0.60				
	Sum	209	0.71				
	0.1*M* NH_4NO_3	587	2.02				

* Of the total K.

† In air-dry plants.

modified to suit soil conditions was tried and wheat seedlings were grown in some of the treated soils for 2 weeks. Similar experiments were made on others with buckwheat seedlings which were grown for 3 weeks. The latter plants have been reported by Haley (13) as also having an avidity for K in culture experiments.

In each case 100 germinated seeds were planted in 100 gm. of air-dried soil in a shallow dish. The seeds were previously germinated so as to insure their viability and uniformity in number.

The soils for these experiments were allowed to air-dry, the germinated seed were planted and a uniform moisture content was afterwards maintained. At the end, the plants were cut close to the ground, washed with distilled

H_2O, air-dried, and weighed. The entire lot from each treatment was moistened with concentrated H_2SO_4 and ashed until the organic matter was destroyed. To the residue, HCl (1:1) was added, the whole was digested on the steam bath and filtered. The filtrate was evaporated with H_2PtCl_6, the residue treated with acid C_2H_5OH, and K determined. These experiments were carried on at the same time as the digestions.

TABLE 8

Madison County soil No. 2—Total K, 9,500 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED By solvents		POTASSIUM (K) EXTRACTED By plants		WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		p.p.m.	*per cent**	*gm.*	*per cent*†	*gm.*	
None	Distilled H_2O	97	1.02	0.0227	2.32	0.98	Buckwheat
	$0.2N$ HNO_3	123	1.29				
	Sum	220	2.31				
	$0.2M$ NH_4NO_3	255	2.68				
S	Distilled H_2O	104	1.09	0.0208	2.17	0.96	
	$0.2N$ HNO_3	116	1.22				
	Sum	220	2.31				
	$0.2M$ NH_4NO_3	202	2.13				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	101	1.06	0.0081	0.97	0.84	
	$0.2N$ HNO_3	125	1.32				
	Sum	226	2.38				
	$0.2M$ NH_4NO_3	200	2.11				
$CaCO_3$	Distilled H_2O	61	0.64	0.0120	1.35	0.89	
	$0.2N$ HNO_3	140	1.47				
	Sum	201	2.11				
	$0.2M$ NH_4NO_3	167	1.76				
S and $CaCO_3$	Distilled H_2O	66	0.69	0.0139	1.60	0.87	
	$0.2N$ HNO_3	132	1.39				
	Sum	198	2.08				
	$0.2M$ NH_4NO_3	163	1.72				
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	Distilled H_2O	59	0.62	0.0017	1.09	0.16	
	$0.2N$ HNO_3	141	1.48				
	Sum	200	2.10				
	$0.2M$ NH_4NO_3	174	1.83				

* Of the total K.

† In air-dry plants.

The results of the solubility tests and plant studies are given in tables 1 to 14 and the other determinations in table 15. In reporting gains or losses, only those amounting to over 5 per cent were considered.

DISCUSSION OF RESULTS AND CONCLUSIONS

This investigation consists mainly of solubility tests of K in 11 Kentucky soils of different types which were subjected to different treatments. The soils were mixed with S or $CaSO_4 \cdot 2H_2O$ and in some instances, $CaCO_3$ was added

TABLE 9

Muhlenburg County soil—Total K, 12,300 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	*per cent**	*gm.*	*per cent†*	*gm.*	
None	Distilled H_2O	22	0.18	0.0202	2.06	0.98	Buckwheat
	0.2N HNO_3	73	0.59				
	Sum	95	0.77				
	0.1M NH_4NO_3	86	0.70				
S	Distilled H_2O	27	0.22	0.0289	2.60	1.11	
	0.2N HNO_3	75	0.61				
	Sum	102	0.83				
	0.1M NH_4NO_3	77	0.63				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	22	0.18	0.0131	1.47	0.89	
	0.2N HNO_3	77	0.63				
	Sum	99	0.81				
	0.1M NH_4NO_3	75	0.61				
$CaCO_3$	Distilled H_2O	17	0.14	0.0130	1.48	0.88	
	0.2N HNO_3	77	0.63				
	Sum	94	0.77				
	0.1M NH_4NO_3	88	0.72				
S and $CaCO_3$	Distilled H_2O	16	0.13	0.0078	0.95	0.82	
	0.2N HNO_3	77	0.63				
	Sum	93	0.76				
	0.1M NH_4NO_3	70	0.57				
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	Distilled H_2O	16	0.12	0.0112	1.29	0.87	
	0.2N HNO_3	80	0.65				
	Sum	96	0.77				
	0.1M NH_4NO_3	84	0.68				

* Of the total K.

† In air-dry plants.

together with either of the above materials. The treated soils were maintained at a uniform moisture content for 4 months, at the temperature of the laboratory, and were frequently stirred. The soluble K was then determined by digesting them in different solvents (distilled H_2O, 0.2N HNO_3 and either 0.1M or 0.2M NH_4NO_3).

As young wheat and buckwheat plants have been reported to have an avidity for soluble K, they were grown in the treated soils for a short time at the end of the 4-month period of digestion and the K in the plant was determined. The plants grew well in all cases, except that on one treated soil of Madison County, No. 2, the buckwheat did not do so well as on the other

TABLE 10

Logan County soil—Total K, 14,600 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	*per cent**	*gm.*	*per cent†*	*gm.*	
None	Distilled H_2O	14	0.10	0.0094	1.07	0.88	Buckwheat
	0.2*N* HNO_3	67	0.46				
	Sum	81	0.56				
	0.2*M* NH_4NO_3	68	0.47				
S	Distilled H_2O	18	0.12	0.0149	1.60	0.93	
	0.2*N* HNO_3	65	0.45				
	Sum	83	0.57				
	0.2*M* NH_4NO_3	66	0.45				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	14	0.10	0.0151	1.51	1.00	
	0.2*N* HNO_3	67	0.46				
	Sum	81	0.56				
	0.2*M* NH_4NO_3	57	0.39				
$CaCO_3$	Distilled H_2O	10	0.07	0.0077	0.90	0.85	
	0.2*N* HNO_3	80	0.55				
	Sum	90	0.62				
	0.2*M* NH_4NO_3	59	0.40				
S and $CaCO_3$	Distilled H_2O	13	0.09	0.0141	1.48	0.95	
	0.2*N* HNO_3	76	0.52				
	Sum	89	0.61				
	0.2*M* NH_4NO_3	67	0.46				
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	Distilled H_2O	9	0.06	0.0103	1.14	0.90	
	0.2*N* HNO_3	74	0.51				
	Sum	83	0.57				
	0.2*M* NH_4NO_3	99	0.68				

* Of the total K.

† In air-dry plants.

samples of this soil. These experiments were used as a further test for liberated K, since it was thought that possibly some of the liberated K might be adsorbed by the soil and hence would not be obtained in solution but nevertheless might be utilized by the plant.

Table 12 shows that all soils gave increases in H_2O-soluble K with the S treatment and 5 out of 7 tested show gains even when $CaCO_3$ was added with S. Only one soil, however, produced more H_2O-soluble K with $CaSO_4 \cdot 2H_2O$, although 3 soils with this material gave an increased amount of K in the NH_4NO_3 digestion.

TABLE 11

Laurel County soil—Total K, 12,200 p.p.m.

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	*per cent**	*gm.*	*per cent*†	*gm.*	
None	Distilled H_2O	49	0.40	0.0092	1.12	0.82	Buckwheat
	$0.2N$ HNO_3	119	0.98				
	Sum	168	1.38				
	$0.2M$ NH_4NO_3	162	1.33				
S	Disilled H_2O	57	0.470	0.0099	1.19	0.83	
	$0.2N$ HNO_3	111	0.91				
	Sum	168	1.38				
	$0.2M$ NH_4NO_3	156	1.28				
$CaSO_4 \cdot 2H_2O$	Distilled H_2O	49	0.40	0.093	1.05	0.89	
	$0.2N$ HNO_3	117	0.96				
	Sum	166	1.36				
	$0.2M$ NH_4NO_3	147	1.20				
$CaCO_3$	Distilled H_2O	29	0.24	0.0150	1.67	0.90	
	$0.2N$ HNO_3	125	1.02				
	Sum	154	1.26				
	$0.2M$ NH_4NO_3	119	0.98				
S and $CaCO_3$	Distilled H_2O	55	0.45	0.0173	1.99	0.87	
	$0.2N$ HNO_3	105	0.86				
	Sum	160	1.31				
	$0.2M$ NH_4NO_3	127	1.04				
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	Distilled H_2O	28	0.23	0.0134	1.46	0.92	
	$0.2N$ HNO_3	126	1.03				
	Sum	154	1.26				
	$0.2M$ NH_4NO_3	124	1.02				

* Of the total K.

† In air-dry plants.

According to table 7, a greater quantity of K was found in wheat grown in Madison No. 1 soil after the $CaSO_4 \cdot 2H_2O$ treatment, and larger amounts of the same element were extracted by H_2O and NH_4NO_3. This indicates that $CaSO_4 \cdot 2H_2O$ may have liberated K in this soil. The results on the other soils, however, generally were not so good with $CaSO_4 \cdot 2H_2O$ as with S.

TABLE 12

Effect of treatments on soluble potassium in soils (tables 1 to 11)

ADDITIONS	NUMBER OF SOILS	
		H_2O-soluble K
S	11	Gain in all, particularly 4 (McCracken, Taylor, Shelby, Laurel)
$CaSO_4 \cdot 2H_2O$	11	Gain in 1 (Madison No. 1); loss in 1 (Fayette)
$CaCO_3$	7	No gain; loss in all, particulary 5 (Graves, McCracken, Taylor, Madison No. 2, Laurel)
S and $CaCO_3$*	7	Gain in 5 (McCracken, Taylor, Madison No. 2, Logan, Laurel). Loss in 1 (Muhlenburg).
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$*	7	No gain; loss in 4 (Graves, Taylor, Muhlenburg, Logan).
		0.2 normal HNO_3-soluble K
S	11	No gain; loss in 5 (Graves, McCracken, Taylor, Madison No. 2, Laurel)
$CaSO_4 \cdot 2H_2O$	11	No gain; loss in 3 (Graves, Franklin, Madison No. 1)
$CaCO_3$	7	Gain in 3 (McCracken, Madison No. 2, Logan); loss in 2, (Graves, Taylor).
S and $CaCO_3$*	7	No gain; loss in 2 (Madison No 2 and Laurel).
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$*	7	Gain in 1 (Taylor); loss in 1 (Logan).
		H_2O-soluble K + 0.2 normal HNO_3-soluble K
S	11	Gain in 2 (Taylor, Muhlenburg)
$CaSO_4 \cdot 2H_2O$	11	No gain; loss in 1 (Franklin)
$CaCO_3$	7	Gain in 2 (McCracken, Logan); loss in others except Muhlenburg.
S and $CaCO_3$*	7	No gain or loss
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$*	7	No gain; loss in 1 (Logan)
		NH_4NO_3-soluble K
S	11	Gain in 4 (Graves, McCracken, Taylor, Franklin); loss in 4 (Fayette, Madison Nos. 1 and 2, Muhlenburg
$CaSO_4 \cdot 2H_2O$	11	Gain in 3 (Graves, McCracken, Madison No. 1); loss in remainder except Franklin.
$CaCO_3$	7	No gain; loss in all except Muhlenburg.
S and $CaCO_3$*	7	Gain in 3 (Taylor, Logan, Laurel); loss in 1 (Muhlenburg)
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$*	7	Gain in 2 (McCracken, Logan); loss in 1. (Taylor).

* Combined treatment compared with $CaCO_3$.

There appears to be no consistent correlation between the amounts of K extracted by the solvent and by the plant except in a few instances. Table 14 shows, however, that those soils which gave an increase of K in the plant, in the majority of instances also show an increase in H_2O-soluble K. As for the other solvents in this table, usually the contrary was true although more agreement with the plant is shown by NH_4NO_3 than by HNO_3. This indicates that the plants obtained the K for their initial growth from that which was soluble rather than absorbed.

TABLE 13

Effect of treatments on potassium in plants grown in treated soils (tables 1 to 11)

ADDITIONS	NUMBER OF SOILS	
	Wheat	
S	7	Gain in 3 (Fayette, Franklin, Madison No. 1) both in per cent and weight; loss in others except McCracken.
$CaSO_4 \cdot 2H_2O$	7	Gain in 3 (McCracken, Franklin, Madison No. 1) in per cent but only in last 2 in weight. Loss in remainder either in per cent or weight.
	Buckwheat	
S	4	Gain in 3 (Muhlenburg, Logan, Laurel) both in per cent and weight; loss in 1 (Madison No. 2).
$CaSO_4 \cdot 2H_2O$	4	Gain in 1 (Logan) both in per cent and weight; loss in remainder either in per cent or weight.
$CaCO_3$	7*	Gain in 1 (Laurel) both in per cent and weight; loss in remainder.
S and $CaCO_3$†	7	Gain in 4 (McCracken, Madison No. 2, Logan, Laurel) both in per cent and weight; loss in 2 (Taylor, Muhlenburg) either in per cent or weight.
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$†	7	Gain in 1 (Logan) both in per cent and weight; loss in remainder.

* There were only 4 samples in which this comparison could be made because wheat was grown in the other 3 untreated soils.

† Combined treatment compared with $CaCO_3$.

The $CaCO_3$ generally had a beneficial effect on the oxidation of S but a depressive one on the soluble K extracted by every solvent except $0.2N$ HNO_3. For instance, in table 15, according to the added S oxidized, the combination of S and $CaCO_3$ showed gains over S in 6 soils, varying from 16 per cent in Laurel County to 3862 per cent in Taylor County. The Logan County sample was the only exception and this showed a loss of 6 per cent. On the other hand, table 12 shows that $CaCO_3$ depressed the solubility of K in distilled H_2O in every soil and the solubility in NH_4NO_3-solution in all except

Muhlenburg County. This is of interest inasmuch as it shows that $CaCO_3$ apparently has contradictory functions, one beneficial, to promote the oxida-

TABLE 14

*Correlation of gains in potassium shown by plants and solubility tests in different treatments of soils (tables 12–13)**

SOIL†	WHEAT	H_2O-SOLUBLE K	0.2 *N* HNO_3-SOLUBLE K	H_2O-SOLUBLE K + 0.2 *N* HNO_3-SOLUBLE K	NH_4NO_3-SOLUBLE K
		S			
Fayette	+	+			+
Franklin	+	+			
Madison No. 1	+	+			
		$CaSO_4 \cdot 2H_2O$			
McCracken	+‡				+
Franklin	+				
Madison No. 1	+	+			+
		S			
	BUCKWHEAT				
Muhlenburg	+	+		+	
Logan	+	+			
Laurel	+	+			
		$CaSO_4 \cdot 2H_2O$			
Logan	+				
		$CaCO_3$			
Laurel	+		+		
		S and $CaCO_3$§			
McCracken	+	+			
Madison No. 2	+	+			
Logan	+	+			+
Laurel	+	+			+
		$CaSO_4 \cdot 2H_2O$ and $CaCO_3$§			
Logan	+				+

* Gains indicated by +. Blank spaces indicate no gain and frequently a loss.

† Only the soils are mentioned in which the plant grown in the treated soil showed a gain both in per cent and weight of potassium.

‡ Only in per cent of potassium.

§ Combined treatment compared with $CaCO_3$.

tion of S to H_2SO_4 which reacts with the soil silicates to form soluble K, and the other detrimental since it seems to increase the adsorption of K by the soil.

Its detrimental effect might be partly explained as due to its neutralization of the acid before the latter can act on the soil, but nevertheless a comparison

TABLE 15

Sulfur oxidized and hydrogen-ion concentration at the end of the experiments

SOIL	ADDITIONS	SULFATE S		ADDED S OXIDIZED		pH
		Initial amount	Final amount	Amount	Per cent of amount added	
		p.p.m.	*p.p.m.*	*p.p.m.*		
Graves County	None	46	121	—	—	6.5
	S	—	197	76	30	6.3
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	6.5
	$CaCO_3$	—	73	—	—	8.5
	S and $CaCO_3$	—	250	177	71	8.4
	$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	—	—	—	—	8.6
Fayette County	None	101	155	—	—	6.3
	S	—	191	36	14	6.3
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	6.3
McCracken County	None	43	41	—	—	6.7
	S	—	75	34	14	6.5
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	6.6
	$CaCO_3$	—	69	—	—	8.4
	S and $CaCO_3$	—	269	200	80	8.3
	$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	—	—	—	—	8.3
Taylor County	None	40	87	—	—	6.3
	S	—	95	8	3	6.3
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	6.3
	$CaCO_3$	—	35	—	—	8.4
	S* and $CaCO_3$	—	360	325	65	8.2
	$CaSO_4 \cdot 2H_2O$* and $CaCO_3$	—	—	—	—	8.3
Shelby County	None	40	67	—	—	6.1
	S	—	78	11	4	6.1
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	6.1
Franklin County	None	53	66	—	—	6.9
	S	—	69	3	1	6.8
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	6.9
Madison County No. 1	None	136	91	—	—	7.0
	S	—	111	20	8	6.9
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	7.0

* The amounts of S and $CaSO_4 \cdot 2H_2O$ added were 500 p.p.m. instead of the usual applications of 250 p.p.m.

TABLE 15—*Continued*

SOIL	ADDITIONS	SULFATE S		ADDED S OXIDIZED		pH
		Initial amount	Final amount	Amount	Per cent of amount added	
		p.p.m.	*p.p.m.*	*p.p.m.*		
Madison County No. 2	None	78	104	—	—	6.1
	S	—	253	149	60	6.0
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	6.1
	$CaCO_3$	—	115	—	—	8.2
	S and $CaCO_3$	—	308	193	77	8.2
	$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	—	—	—	—	8.2
Muhlenburg County	None	55	78	—	—	6.4
	S	—	185	107	43	6.1
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	6.2
	$CaCO_3$	—	43	—	—	8.3
	S and $CaCO_3$	—	188	145	58	8.1
	$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	—	—	—	—	8.2
Logan County	None	47	61	—	—	6.3
	S	—	234	173	69	6.0
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	6.3
	$CaCO_3$	—	66	—	—	8.5
	S and $CaCO_3$	—	229	163	65	8.2
	$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	—	—	—	—	8.4
Laurel County	None	41	44	—	—	6.3
	S	—	156	112	45	6.2
	$CaSO_4 \cdot 2H_2O$	—	—	—	—	6.2
	$CaCO_3$	—	18	—	—	8.4
	S and $CaCO_3$	—	148	130	52	8.2
	$CaSO_4 \cdot 2H_2O$ and $CaCO_3$	—	—	—	—	8.2

of the combined S and $CaCO_3$ treatments with the corresponding untreated soils seems to show that the base has increased the capacity of the soil to adsorb the soluble K as shown both in the H_2O and NH_4NO_3 digestions.

In the majority of treatments without $CaCO_3$, the amount of K soluble in NH_4NO_3 solution was larger than the combined amount obtained by distilled H_2O and 0.2*N* HNO_3, whereas with this base present, it was less. Moreover, with the same solvent, when $CaCO_3$ was present in the treatment, the K obtained was generally less than that extracted from the untreated soil.

There was very little variation in the hydrogen-ion concentration in the different treatments of the same soil, according to the method used, except when $CaCO_3$ was added. The amounts of S oxidized in some soils indicate that considerable quantities of acid were produced.

The fact that all samples to which $CaCO_3$ was added were rendered alkaline and all except the Logan County sample oxidized larger quantities of S, indicates that the S organims to which an alkaline medium is favorable, were predominant in these soils.

REFERENCES

(1) Ames, J. W., and Boltz, G. E. 1919 Effect of sulfofication and nitrification on potassium and other soil constituents. *Soil Sci.* 7: 183–195.

(2) Ames, J. W., and Richmond, T. E. 1918 Effect of sulfofication and nitrification on rock phosphate. *Soil Sci.* 6: 351–364.

(3) Ames, J. W., and Simon, R. H. 1924 Soil potassium as affected by fertilizer treatment and cropping. Ohio Agr. Exp. Sta. Bul. 379.

(4) André, G. 1913 Deplacement de la potasse contenue dans certaines roches feldspathiques par quelques substances employées comme engrais. *Compt. Rend. Acad. Sci. (Paris)* 157: 856–8. (Original not seen—Abs. in *Chem. Abs.*, 8: 546.)

(5) Association of Official Agricultural Chemists 1907 Proceedings of the 24th Annual Convention. *U. S. Dept. Agr. Bur. Chem. Bul.* 116: 90(d).

(6) Bradley, C. E. 1910 The reaction of lime and gypsum on some Oregon soils. *Jour. Indus. and Engin. Chem.* 2: 529–530.

(7) Briggs, L. J., and Breazeale, J. F. 1917 Availability of potash in certain orthoclase-bearing soils as affected by lime or gypsum. *Jour. Agr. Res.*, 8: 21–28.

(8) Brown, P. E., and Warner, H. W. 1917 The production of available phosphorus from rock phosphate by composting with sulfur and manure. *Soil Sci.* 4: 269–282.

(9) Burgess, P. S. 1922 Studies on a drained marsh soil unproductive for peas. *Univ. Cal. Pubs. Agr. Sci.* 4: 339–96.

(10) Clark, W. M. 1920 The Determination of Hydrogen Ions. Baltimore, Md.

(11) Dumont, J. 1904 Action des composes calciques sur la mobilisation de la potasse du sol. *Bul. Soc. Nat. Agr. France* 64: 379–84. (Original not seen—Abs. in *Exp. Sta. Rec.*) 16: 656–657.

(12) Fraps, G. S. 1916 Effects of additions on the availability of soil potash and the preparation of sugar humus. Texas Agr. Exp. Sta. Bul. 190.

(13) Haley, D. E. 1924 A biological measurement of the availability of potassium in soils. *Pa. Agr. Exp. Sta. Bul.* 188: 8.

(14) Hilgard, E. W. 1906 Soils, their Formation, Properties, Composition and Relations to Climate and Plant Growth in the Humid and Arid Regions. New York and London.

(15) LeClerc, J. A., and Breazeale, J. F. 1911 Translocation of plant food and elaboration of organic plant material in wheat seedlings. U. S. Dept. Agr. Bur. Chem. Bul. 138.

(16) Lipman, C. B., and Gericke, W. F. 1918. Does $CaCO_3$ or $CaSO_4$ treatment affect the solubility of the soil's constituents. *Univ. Cal. Pubs. Agr. Sci.* 3: 271–282.

(17) Lipman, J. G., McLean, H. C., and Lint, H. C. 1916 The oxidation of sulfur in soils as a means of incieasing the availability of mineral phosphates. *Soil Sci.* 1: 353–359.

(18) Lyon, T. L., and Buckman, H. J. 1922 The Nature and Properties of Soils. New York.

(19) McCall, A. G., and Smith, A. M. 1920 Effect of manure-sulfur composts upon the availability of the potassium of greensand. *Jour. Agr. Res.* 19: 239–256.

(20) McMiller, P. R. 1918 Influence of gypsum upon the solubility of potash in soils *Jour. Agr. Res.* 14: 61–66.

(21) MORSE, F. W., AND CURRY, B. E. 1909 The availability of the soil potash in clay and clay loam soils. *N. H. Agr. Exp. Sta. Bul.* 142: 37–56.

(22) RUDOLFS, W. 1922 Sulfur oxidation in inoculated and uninoculated greensand mixtures and its relation to the availability of potassium. *Soil Sci.* 14: 307–19.

(23) SHEDD, O. M. 1919 Effect of oxidation of sulfur in soils on the solubility of rock phosphate and on nitrification. *Jour. Agr. Res.* 18: 329–345.

(24) STORER, F. H. 1888 Agriculture in Some of its Relations with Chemistry, v. 1. New York.

EFFECT OF GROWING LEGUMES UPON SUCCEEDING CROPS

F. LÖHNIS[1]

Bureau of Plant Industry, Department of Agriculture

Received for publication October 1, 1925

LITERATURE

It has been known since ancient times that legumes exert a beneficial effect upon the succeeding crops. This knowledge has played an important part in the development of crop rotations in Europe, and early American authors (3, 28) have likewise emphasized the good after-effect of clover. But how this beneficial effect is being accomplished has not been fully explained.

There are several causes that have to be considered: (*a*) an increase in the soil nitrogen by the crop residues, (*b*) an increase of potash and phosphate in the surface soil due to the transportation of these substances from the subsoil by deep-rooted legumes, (*c*) a change in soil reaction, (*d*) an improved physical structure of the soil, (*e*) suppression of weeds, (*f*) increased bacterial activities.

Naturally, most attention has been paid to the increase in soil nitrogen. Long continued experiments made at the Rothamsted Experiment Station have shown very conspicuous accumulations of nitrogen under legumes (7), and numerous other tests have given similar results. On the other hand, several authors have strongly emphasized, that if only stubble and roots are left on the field no increase in nitrogen takes place (9, 16, 27, 34, 35, 36). Investigations made at the Wyoming Station (4) have demonstrated that alfalfa cut for hay exerted its characteristic beneficial after effect upon wheat and oats although the roots were free from nodules, and therefore no symbiotic nitrogen fixation could have taken place. Therefore, it is very probable that wherever an increase becomes noticeable in the soil nitrogen under and after legumes, this is much less due to the nitrogen fixation by the bacteria in the root nodules, than to the action of non-symbiotic soil organisms assimilating elementary nitrogen.

The lifting of potash and phosphate from the subsoil by deep rooted legumes is another point upon which not much accurate information is available. At the Kansas Experiment Station uninoculated soybeans that were free from nodules, increased the following wheat crop very distinctly. The investigators were of the opinion that this effect was partly due to the potash and phosphate liberated from the decaying roots (5). More recently this point was emphasized in explaining results obtained in Germany with stubble and roots of lupines (10).

The increase in soil acidity to which several authors have ascribed occasional unsatisfactory effects of green manuring (30, 31), is probably of very little importance in this case (17) as well as the after-effect of harvested legumes. The crop rotation experiments carried on at the Rhode Island Station have shown that in certain cases the acidity factor may be of considerable importance (13), but hardly anything is known about the behavior of the different legumes. A few data obtained at the Cornell Station have been explained as

[1] The writer is indebted to F. M. Scales and N. R. Smith for the supervision of field and greenhouse experiments, to A. P. Harrison and K. S. Markley for the analyses and calculations, and to F. L. Goll for the drawing of the graph.

indicating an increased soil acidity after harvested legumes (25). The beneficial after-effect of legume growth is not reduced, however, by liming, as will be shown presently. Therefore, increased soil acidity can not be of considerable importance in this case.

The possibility of improvement of physical soil structure under a heavy growth of legumes, which has always attracted the attention of careful observers (12, 14, 28), deserves the full attention of the practical farmer, at least in regions with insufficient rainfall. The growing crop of legumes needs much water; therefore, if rain is lacking this beneficial effect can not be expected, and fallowing will be preferable.

The suppression of weeds by the growing legumes is another point of great practical importance. Naturally, this benefit will be fully realized only if a close, heavy development of legumes is secured. Therefore, accurate knowledge of the kind of legumes best suited to the particular soil and climate is again much to be desired. The water saved for the succeeding crop by the suppression of weeds is undoubtedly in many cases an important, though frequently overlooked, contributing cause to the beneficial influence exerted by the preceding legumes.

Increased bacterial action, especially intensified nitrification under legumes, also deserves careful investigation (23, p. 767). As early as in 1895 the nitrate accumulation under legumes was strongly emphasized by Laws and Gilbert (18, p. 130). Experiments made at Rothamsted in 1885, which were published in 1909 (7) showed nitrate present in large amounts under white and sweet clovers, whereas it was almost absent under alfalfa. Lyon and his associates noticed high nitrification under alfalfa and clover, whereas it was low under soybeans (24, 25). LeClair (19) found strong nitrifying efficiency under cowpeas, and Holtz and Singleton (15) again under alfalfa and sweet clover.

Conspicuous increases in the total number of soil bacteria and in the carbon dioxide produced by them have also been observed repeatedly. Two-to-four-fold increases have been recorded in experiments with cowpeas (19). According to Stoklasa (33) higher counts and stronger CO_2 production are to be expected under alfalfa and red clover than under cereals.

That especially non-symbiotic nitrogen fixing bacteria are stimulated in their development by the legumes was pointed out by Liebscher and by Beijerinck about 20 and 30 years ago (23, p. 770), but nothing more definite has become known since then. Field experiments made in West Virginia (2) gave apparent gains in nitrogen of 78 pounds per acre and year, when in a 16-year rotation cowpeas or clover were grown four times and phosphate and potash were applied as fertilizers. Since the surface growth of the legumes was removed, the results obtained would constitute a definite proof of strong nitrogen fixation in the soil, were it not for the uncertainty of soil nitrogen determinations.

EXPERIMENTAL RESULTS

A combination of field, greenhouse, and laboratory experiments, was adopted to secure more complete information on the effect of growing legumes upon succeeding crops. The results of field experiments are of greatest practical value, but considerable uncertainty is frequently attached to them. Experiments in the greenhouse allow a much better control of all conditions but as these conditions and the results are more or less different from those obtainable in the field, the underlying causes of the results are often left in doubt. Suitably arranged laboratory experiments are therefore necessary. A careful coordination of the different lines of work can do much to enhance the advantages and to reduce the disadvantages of the three ways of investigating problems in soil bacteriology.

Field experiments

An area of about three acres at Arlington Experimental Farm, that had been kept permanently under grass, was selected in 1914 and laid out into 120 plots of slightly varying size. Field I consisted of 48 plots, each $\frac{1}{54}$ acre. Three sets of 24 plots each, wherein every plot was $\frac{1}{60}$ acre, were designated fields II A, II B, and III. The grass was cut twice on the marked plots in 1914, and after the weights obtained had demonstrated a fair uniformity of the soil, the permanent field experiments were started in the fall. In order to secure definite information upon the suitability of the land, no fertilizer or manure was applied to the 1915 crop.

Chemical and biological tests indicated that the rather heavy clay soil of the field, contains much potash (1.4 per cent K_2O) and phosphate (0.25 per cent P_2O_5). Neither potash nor phosphate, nor both combined, either with or without mineral or organic nitrogen, exerted any effect upon the crops grown on small test plots at the ends of the fields.

The original soil reaction was pH 6.4, but in view of the physical structure of the soil the whole area received an application of slaked lime before the sod was broken and this treatment was repeated regularly as noted below ($1\frac{1}{2}$ tons per acre for an eight-year rotation).

The soil of field I is somewhat lighter than that of fields II and III. The original nitrogen content was 0.07 per cent on field I, and 0.10 per cent on fields II and III.

The crop rotations, as planned originally, were as follows:

Field I	*Fields II and III*
1. Wheat [Hairy Vetch and Rye]	1. Wheat [Cowpeas, Soybeans]
2. Soybeans and Cowpeas, or Beets	2. Potatoes +
3. Corn	3. Wheat [Cowpeas, Soybeans]
4. Potatoes +	4. Corn
5. Oats [Hairy Vetch and Rye]	5. Corn +
6. Soybeans and Cowpeas, or Beets	6. Oats [Clover and Grass]
7. Corn	7. Clover and Grass
8. Potatoes +	8. Clover and Grass

The main difference between the rotations is in the crop succession. On field I the grain crops are separated from each other by legumes or potatoes, as is customary in Europe; on fields II and III the grain crops follow each other, as is frequently practised in America. One-year wheat had to be replaced by another year of potatoes on field I, and instead of clover, soybeans and cowpeas were grown. The catch crops after wheat, given in brackets, were likewise different. The crops receiving stable manure are marked +. Lime was regularly applied to corn on field I, and to oats on fields II and III. On four plots of field I the legumes were replaced by beets.

Since the topography of the area did not permit a further extension of the experimental plots on field I, only four crops could be grown simultaneously, whereas on fields II and III the complete rotation was repeated each year. On account of the uniform distribution of the various crops in the first rotation,

TABLE 1

*Nitrogen (pounds per acre) in crops harvested 1915 to 1924**

All (main) crops = averages of 6 years of cereals and potatoes, 2 years of legumes, beets, or clover and grass

FIELD	TREATMENT	CROPS	1915	1916	1917	1920	1921	1922	1923	1924	8 YEARS TOTAL
I	No nitrogen	Cereals and potatoes	20.42	32.20	28.56	23.61	20.15	14.61	39.34	32.91	211.80
		Beets	45.50	78.62	29.64	21.74	Failed	67.08	37.96	31.32	311.86
		All crops	27.08	43.63	28.87	23.21	15.00	28.50	39.05	34.51	239.85
	Mineral nitrogen	Cereals and potatoes	18.81†	38.34	57.60	36.83	21.36†	19.40	46.80	44.19	283.33
		Beets	42.38†	108.76	52.91	19.11	Failed	77.70	57.33	37.80	395.99
		All crops	24.91†	56.41	56.49	32.35	15.90†	34.47	49.61	42.28	313.42
	Legumes	Cereals and potatoes	19.71	36.98	42.93	38.26	22.86	29.97	47.25	41.40	279.36
		Legumes	55.38	150.54	105.56	52.39	102.60	72.90	86.21	66.15	691.73
		Main crops	27.62	66.38	59.14	41.25	42.40	40.36	56.70	47.17	381.02
		Catch crops	.....	43.16	53.04	76.28	57.78	32.94	47.79	....†	310.99
	Legumes and fresh stable manure	Cereals and potatoes	18.38†	41.22	49.95	41.70	28.44	30.51	67.89	50.58	328.67
		Legumes	61.56	138.08	132.35	54.65	128.09	84.08	93.61	73.17	765.59
		Main crops	28.96†	64.95	70.03	44.60	52.94	43.58	73.77	55.74	434.57
		Catch crops	.....	41.18	58.24	68.85	60.62	54.22	71.25	.....†	354.36

I	Legumes and 5 weeks old stable manure	Cereals and potatoes	20.43†	40.41	53.01	41.65	26.91	28.45	66.25	48.06	325.17
		Legumes	57.05	154.31	134.87	53.51	130.06	103.00	86.59	75.87	795.26
		Main crops	29.63†	68.39	72.92	46.45	52.31	46.65	70.80	56.14	443.29
		Catch crops	.....	35.02	67.01	64.13	58.56	53.87	70.71	.....†	349.30
	Legumes and 10 weeks old stable manure	Cereals and potatoes	19.80†	42.30	55.53	47.62	26.59	27.58	74.18	50.85	344.45
		Legumes	63.35	153.23	124.39	56.65	130.84	108.60	78.65	72.90	788.61
		Main crops	30.40†	69.50	71.96	49.51	52.26	47.47	75.48	56.01	452.59
		Catch crops	.....	36.26	67.72	68.18	59.76	55.40	70.28	.....†	357.60
II/III	No nitrogen	Cereals and potatoes	43.93	51.73	49.21	28.73	25.71	32.42	50.84	47.00	329.57
		Clover and grass	36.90	130.16	163.60	45.59	33.82	13.17	8.00	58.70	489.94
		All crops	42.18	70.85	77.81	32.45	27.74	27.61	40.13	49.92	368.69
	Mineral nitrogen	Cereals and potatoes	44.73†	62.23	56.83	33.70	29.45	31.66†	51.72	53.73	364.05
		Clover and grass	33.20†	143.91	172.36	46.65	34.87	11.09†	9.00	56.11	807.19
		All crops	41.85†	82.65	85.71	36.94	30.80	24.52†	41.04	55.07	398.58
	Legumes	Cereals and potatoes	41.07	55.26	50.63	37.46	28.81	30.81	46.01	45.43	335.48
		Clover and grass	37.80	100.46	161.58	44.49	35.72	12.28	8.90	65.13	466.36
		Main crops	40.25	66.56	78.37	41.72	30.54	26.18	36.74	50.36	370.72
		Catch crops	43.00	47.92	Failures		Failures		131.40	65.60	287.92

* Exclusive of crops of 1918 and 1919.

† No mineral nitrogen and no stable manure in 1915; no mineral nitrogen on field I in 1921, on field II/III in 1922; no catch crop on field I in 1924 because of change in rotation.

this arrangement could be made without endangering the reliability of the results obtained.

The differential treatment on field I was as follows:

Two strips running across the four differently planted strips received no nitrogenous manure, and two received nitrate and ammonium sulfate equivalent to an average application of 30 pounds nitrogen per acre and year (240 pounds for the full rotation). On these four strips beets were planted instead of legumes. On the remaining eight strips, legumes were grown as source of nitrogen and six of them received in addition, a small application of cow manure (equal to 15 tons containing approximately 160 pounds nitrogen per acre, for the eight-year rotation). This manure was spread on two strips approximately 10 weeks before planting; on two, about 5 weeks later; and on the remaining two strips, immediately before they were prepared for the planting of potatoes.

Fields II A, II B, and III were divided in thirds across the full rotation. Clover was grown on all plots, and stable manure (in this case horse manure in the same quantity as above) was applied to all plots. One crosswise strip received no further treatment; on another one, nitrate and ammonium sulfate were applied as on field I; and on the third strip, catch crops were grown after wheat. After cowpeas and soybeans had failed repeatedly (in 1917 and 1920) as catch crops sweet clover was tried (in 1921 and 1922), but it likewise proved unsatisfactory. Therefore, hairy vetch mixed with rye was adopted in this case (in 1923 and 1924).

The plan as developed for field I made it possible to observe how mineral nitrogenous fertilizers, the growth of legumes, and the earlier or later application of stable manure would increase the crops above those grown without manuring. On fields II and III on the other hand, all plots were under the influence of clover growth and the application of horse manure, and it remained to be seen what further increases could be secured by the additional use of mineral nitrogen or by the growth of catch crops.

The clover was regularly harvested on all plots, but the plots of fields II and III bearing catch crops, and all plots planted to legumes on field I were equally divided in each case. On one-half of each plot the surface growth was removed, on the other half it was plowed under.

As these experiments had to be stopped temporarily during the war period, all plots that should not get the benefit of leguminous crops were laid down to grass, and on the other plots a mixture of cowpeas and soybeans was sown each year and all growth was removed as hay. Accordingly, no crops are recorded for 1918 and 1919 in table 1. With the exception of the legume strips, the crops harvested in 1920 were as low as, or lower than, those of 1915, especially on fields II and III.

As the first crop in 1915 received neither stable manure nor mineral nitrogen, and as the mineral nitrogen was omitted on field I in 1921 and on fields II and III in 1922, the total nitrogen applied to the full rotations was 180 pounds mineral nitrogen per acre (instead of 240 pounds) and 140 pounds in form of stable manure (against 160 pounds as planned).

As far as the main crops are concerned, wheat started low with 7 bushels per acre, but rose soon to over 30 bushels; corn began with approximately 40 bushels per acre and was brought up to 50 to 60 bushels average; oats gave at first 20, later 60 bushels but the crop was more or less a failure in most years; potatoes were likewise unsatisfactory (with 80 to 150 bushels per acre); clover, too, gave good returns in two or three years only; whereas cowpeas, soybeans, and hairy vetch made invariably a good, or even excellent, growth.

The nitrogen data collected in table 1 give a clear picture of the success of the different treatments. In each case they are calculated in pounds per acre, so as to eliminate the disturbing influence of the varying number and size of plots to be compared.

Several facts stand out very clearly. The first nitrogen returns (in 1915) in cereals and potatoes, show that the average initial figures for all plots on field I, and on fields II and III, respectively, are as uniform as can be expected. The figures for fields II and III are twice as high as those for field I because of

TABLE 2

Nitrogen return in eight years

(Pounds per acre)

CROPS	NITROGEN					
	Above unmanured plots				Above legume plots	
	Mineral nitrogen*		Legumes	Legumes and stable manure†		
	lbs.	*per cent*	*lbs.*	*lbs.*	*lbs.*	*per cent*
Cereals and potatoes	71.5	39.7	67.2	121.0	53.8	38.4
Main crops	73.6	40.3	141.2	203.6	62.4	44.5
Catch crops	....	....	311.0	353.8	42.8	30.5
Total nitrogen return	73.6	40.3	452.2	557.4	105.2	75.0

* 180 pounds N.

† 140 pounds N.

the differences in the quality of the soil. The data for 1923 and 1924 demonstrate that, despite the poorer quality of the soil on field I, the more suitable crop succession has raised the productivity to the same height as on fields II and III, and even higher on the plots that received organic manures. Already in 1916 and 1917 the effects of the different treatments were almost as well defined as they were again in 1923 and 1924, but the interruption caused a break which reduced to some extent the total figures for the eight years' rotations. Nevertheless, the superiority of the combined effect of growth of legumes and application of stable manure is very marked. The total figures for the respective plots on field I are almost identical with those for field II, where clover was grown on all plots for two years and stable manure, was applied uniformly. The plots on field I left without manure, as well as those receiving nothing but mineral

nitrogen or having only the benefit of legumes but no stable manure, are obviously inferior. No additional effect of the leguminous catch crops is noticeable on fields II and III, partly because of the poor growth of these crops and partly because of the predominating influence of the clover and of the stale manure. Because of the last named factor, the mineral nitrogen, too, has caused a very small crop increase.

Soybeans, cowpeas, and hairy vetch gave very regular and satisfactory nitrogen returns on field I (over 1,000 pounds nitrogen per acre for the full rotation), whereas the red clover on fields II and III failed almost completely in four out of eight years. Cowpeas and soybeans planted after wheat on fields II and III were likewise not reliable, (failures in 1917 and 1920); sweet clover proved equally unsatisfactory (in 1921 and 1922) and was therefore replaced by hairy vetch in 1923 and 1924.

The nitrogen recovered in the crops from the mineral and from the organic fertilizers applied on field I has been calculated in table 2.

As far as the main crops are concerned, a 40 per cent return is distinctly unsatisfactory for nitrate and ammonium sulfate, especially if practically the same return is obtained with stable manure. The 30 per cent return in the catch crops, however, raises the total for stable manure to 75 per cent, an unusually high figure, which shows the exceptional value of small applications of animal manure as well as the good effect of cover crops in preventing nitrogen losses by leaching. It might be concluded that the plots with mineral nitrogen would have given a better return if cover crops had been grown on them, too. To some extent this conclusion may be correct. But in view of the complete availability of the mineral nitrogen it is still more probable that the low returns are mainly due to the lack of organic matter in these plots and to the resulting insufficient carbon dioxide supply to the growing plants. That the percentage returns were markedly higher during the first part of the rotation—1916 to 1919: 20:60 per cent; 1922 to 1924: 20 per cent—points in the same direction.

The increase in nitrogen in cereals and in potatoes grown on the legume plots is about as high as that caused by the application of nitrate and ammonium sulfate. A percentage calculation is impossible because it is not known how much nitrogen has been added to the soil by the nitrogen-fixing bacteria of the legumes. But the 500 pounds (approximately) that have been harvested on these plots in eight years in excess of the 240 pounds on the unfertilized plots leaves no doubt that a vigorous bacterial action has taken place. The non-legume crops grown after legumes, have increased in all cases where the surface growth of the legumes has been removed. In only two years (1916 and 1917) did the plowing under of the vines give a little additional effect (12 and 15 per cent, respectivelv, above that secured from stubble and roots), which was almost within the limits of the fluctuations caused by inequalities of the soil.

If the total nitrogen in the stubble and roots of the legumes, is calculated at 250 pounds per 1,000 grounds surface growth, the percentage nitrogen return in cereals and potatoes grown on the legume plots would be approximately 27

per cent. This figure would fit very well those obtained in other experiments on the nitrogen availability in stubble and roots of legumes, but it is more probable that the increase in nitrogen return is not exclusively due to the decomposition of these crop residues.

Nitrogen analyses of the differently treated soil on field I made at the end of the rotation, gave the following average results: No manure, 0.055 per cent; legumes, 0.065 per cent; mineral nitrogen, 0.060 per cent; legumes and manure, 0.074 per cent.

In 1914 the average nitrogen content of this soil was 0.07 per cent. Accordingly, it seems as if the plots left without organic manure have lost a considerable amount of their nitrogen, whereas those receiving organic manures have remained practically constant, and that the large quantities of nitrogen harvested in the legumes have been drawn mainly from the air.

No marked change was noticeable, for fields II and III started with 0.1 per cent in 1914, and contained at the end of the rotation 0.091 per cent on the plots that received no other manure than stable manure and clover residues, and 0.099 per cent on those receiving in addition mineral nitrogen and leguminous catch crops, respectively.

Greenhouse experiments

In 1915 wheat was grown on some of the pots and peas on the remainder.[2] After harvesting, the pea vines, and in some cases their roots, were transferred to the wheat pots, so that comparative tests could be made. For further comparison, dried green manure was likewise used on the poor soil. Simultaneously on both soils, corn, kafir, and milo were grown, which were followed on the rich soil by another crop of corn. The nitrogen applied in the pea tops was 252 mgm. per pot on the poor soil and 410 mgm. on the rich soil. The nitrogen in the roots was not analyzed because the material available was just sufficient for the pot tests.

The data secured are shown in table 3.

The crops of corn, kafir, and milo after peas were not much higher than after wheat, although certain quantities of nitrogen had been removed in the wheat crops (30 mgm. on poor soil, 165 mgm. on rich soil). The increases due to the application of pea tops, either fresh or dry, are in the poor soil somewhat higher after peas than after wheat, whereas there is no marked difference on the rich soil. The pea pots on the rich soil, from which tops as well as roots were carefully removed, gave almost exactly the same returns as the wheat pots, where the crop residues were left. Pea soil plus pea residues produced a higher corn crop than pea soil without residues, but practically the same quantities of kafir and milo. The transferred pea tops and roots together produced about the same crops (dry weight) as the pea tops turned under after

[2] For the description of the pot tests, see the preceding article on "Nitrogen availability of green manures," in *Soil Sci.* 22: 253–290.

wheat, although the nitrogen content is markedly higher. Corn on poor soil returned 10 per cent nitrogen after wheat and 15 per cent after peas; kafir and

TABLE 3

Effect of pea growth, pea tops, and roots

PREVIOUS CROP	TREATMENT		AVERAGE DRY WEIGHTS PER POT			N AVERAGE PER POT		
			Corn	Kafir	Milo	Corn	Kafir	Milo
			gm.	*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*
				Poor soil				
After wheat (30 mgm. N)	Wheat stubble only		7.0	5.0	5.6	44.6	47.1	43.1
	Dried pea tops added		10.5	7.1	7.4	66.6	58.2	60.7
	Fresh pea tops added		11.4	5.9	6.0	69.4	48.4	46.2
	Maximum increase by pea tops		4.4	2.1	1.8	24.8	11.1	17.6
After peas (252 mgm. N)	Pea tops and roots		18.6	9.0	9.2	98.6	71.5	64.4
	Pea stubble and roots		10.2	5.5	7.0	59.2	45.3	49.0
	Increase by tops		8.4	3.5	2.2	39.4	26.2	15.4
				Rich soil				
After wheat (165 mgm. N)	Wheat stubble only	1. Crop	14.4	2.7	3.1	145.8	43.6	45.0
		2. Crop	12.8	15.8	14.5	155.9	180.3	164.0
		Total	27.2	18.5	17.6	301.7	223.9	209.0
	Pea tops added	1. Crop	43.7	4.2	7.2	326.3	68.6	93.5
		2. Crop	14.3	24.3	23.0	139.6	255.3	220.8
		Total	58.0	28.5	30.2	465.9	323.9	314.3
		Increase by tops	30.8	10.0	12.6	164.2	100.0	95.3
	Pea tops and roots added	1. Crop	45.8	5.1	7.0	351.6	104.6	109.5
		2. Crop	14.3	28.1	23.8	150.1	295.5	267.6
		Total	60.1	33.2	30.8	501.7	400.1	377.1
		Above tops	2.1	4.7	0.6	35.8	76.2	61.8
After peas (410 mgm. N)	All residues removed	1. Crop	19.6	4.3	4.8	190.0	64.4	61.9
		2. Crop	11.3	15.7	14.7	117.5	170.4	167.0
		Total	30.9	20.0	19.5	307.5	234.8	228.9

TABLE 3—*Concluded*

PREVIOUS CROP	TREATMENT		AVERAGE DRY WEIGHTS PER POT			N AVERAGE PER POT		
			Corn	Kafir	Milo	Corn	Kafir	Milo
			gm.	*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*
Rich soil—continued								
After peas (410 mgm. N)—Continued	Pea stubble and roots	1. Crop	25.8	4.6	6.8	263.6	61.9	85.0
		2. Crop	12.1	16.1	14.8	127.1	174.4	146.2
		Total	37.9	20.7	21.6	390.7	236.3	231.2
		Increase by roots	7.0	0.7	1.1	83.2	1.5	2.3
	Pea tops and roots	1. Crop	56.3	7.9	9.6	455.5	124.9	141.6
		2. Crop	13.7	24.1	22.6	140.9	204.5	189.7
		Total	70.0	32.0	32.2	596.4	329.4	331.3
		Increase by tops	32.1	11.3	10.6	205.7	93.1	100.1

milo gave still lower results. Rich soil furnished the following data for both crops:

	PER CENT N RECOVERED IN		
	Corn	Kafir	Milo
After wheat	40	24	25
After peas	50	23	24

The effect of growing legumes upon succeeding crops is undoubtedly not exclusively due to the manuring effect of their residues. Five other possible influences have to be considered, viz., increase of available potash and phosphate in the surface soil, slight increases in soil acidity, improved physical structure of the soil, suppression of weeds, and intensified bacterial action.

Crop rotation tests made in the greenhouse permitted the exclusion of all these possibilities except the last one. Regular alternating applications of potassium phosphate and of calcium carbonate supplied a surplus of available phosphate and potash and counteracted any possible increase in soil acidity.[3] No improvement in the physical structure of the soils took place in the pots. Every two years, the pots were emptied and the soil was thoroughly stirred. In the meanwhile, however, the texture, especially of the heavy clay soil, which was extremely poor in organic matter, became very bad.

[3] pH determinations made in 1925 gave for the poor soil 7.4 to 7.6, for the rich soil 6.6 to 6.8.

TABLE 4

Crop rotation experiments

Average dry weights per pot

DATE	CROP SUCCESSION	MISCELLANEOUS CROPS								
		None	Wheat	Rye	Oats	Corn	Field pea	Hairy vetch	Cow-pea	Soy-bean
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
	Poor soil									
1920, September–November	1. Miscellaneous	....	4.2	3.4	3.5	19.0	20.4	7.8	10.7	11.4
November–February	2. Oats	5.6	2.6	2.4	2.3	2.7	7.1	5.5	4.6	2.9
1921, February–May	3. Miscellaneous	....	2.1	1.7	2.3	4.9	17.7	18.5	22.7	15.2
May–July	4. Corn	7.5	6.8	6.7	6.5	7.0	20.5	17.6	14.0	8.8
October–December	5. Corn	9.2	10.2	10.3	9.9	10.7	11.5	10.5	9.4	10.0
1922, January–April	6. Miscellaneous	....	3.8	3.3	4.6	12.9	16.7	15.2	13.9	20.9
April–June	7. Corn	7.9	8.2	8.0	7.7	8.1	9.3	13.1	10.4	10.3
1920–22	(4) Corn (oats) crops	30.2	27.8	27.4	26.4	28.5	48.4	46.7	38.4	32.0
	(3) Miscellaneous crops	....	10.1	8.4	10.4	36.8	54.8	41.5	47.3	48.5
	Total	30.2	37.9	35.8	36.8	65.3	103.2	88.2	85.7	80.5
1923, November–January	1. Corn	13.3	12.8	12.8	13.2	13.1	13.6	13.5	13.4	13.1
1924, February–May	2. Miscellaneous	....	3.6	4.1	7.3	8.4	49.8	38.6	28.6	15.6
June–July	3. Corn	8.4	6.4	6.4	6.1	7.0	19.4	24.5	14.2	8.2
September–October	4. Corn	8.0	7.8	7.7	8.9	8.2	10.1	9.9	9.4	7.7
November–February	5. Miscellaneous	....	1.6	1.8	2.0	1.9	5.9	4.9	6.3	4.2
1925, March–April	6. Corn	5.9	3.7	3.7	3.5	4.7	12.6	8.3	8.8	4.9
May–June	7. Corn	3.4	3.7	3.5	3.9	3.8	4.3	4.3	4.0	3.4
1923–25	(5) Corn crops	39.0	34.4	34.1	35.6	36.8	60.0	60.5	49.8	37.3
	(2) Miscellaneous crops	....	5.2	5.9	9.3	10.3	55.7	43.5	34.9	19.8
	Total	39.0	39.6	40.0	44.9	47.1	115.7	114.0	84.7	57.1

Rich soil

1923, November–January	1. Corn	14.4	13.6	13.9	13.5	13.7	13.6	13.4	13.4	13.4
1924, February–May	2. Miscellaneous	....	8.0	7.0	11.5	17.3	37.4	28.6	21.6	7.2
June–July	3. Corn	17.4	10.2	10.7	9.6	10.4	27.2	28.8	21.9	12.9
September–October	4. Corn	6.9	6.9	6.6	6.9	6.2	8.0	7.6	7.9	5.7
November–February	5. Miscellaneous	....	2.0	2.0	2.4	1.4	6.7	4.5	5.5	4.0
1925, March–April	6. Corn	8.9	5.5	5.7	5.7	8.5	15.9	11.8	11.0	6.0
May–June	7. Corn	5.0	5.1	5.2	5.2	4.8	5.9	5.9	5.2	5.1
1923–25	(5) Corn crops	52.6	41.3	42.1	40.9	43.6	70.6	67.5	59.4	43.1
	(2) Miscellaneous crops	....	10.0	9.0	13.9	18.7	44.1	33.1	27.1	11.2
	Total	52.6	51.3	51.1	54.8	62.3	114.7	100.6	86.5	54.3

TABLE 5

Crop rotation experiments

Average nitrogen returns per pot

DATE	CROP SUCCESSION	MISCELLANEOUS CROPS								
		None	Wheat	Rye	Oats	Corn	Field pea	Hairy vetch	Cow-pea	Soy-bean
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
					Poor soil					
1920, September–November	1. Miscellaneous	.....	55.8	51.2	53.8	173.1	694.5	307.4	380.2	243.9
November–February	2. Oats	60.0	42.6	41.0	36.8	41.9	103.7	69.9	63.9	46.7
1921, February–May	3. Miscellaneous	.....	29.4	27.5	30.8	37.2	584.1	573.5	703.7	408.3
May–July	4. Corn	52.5	54.4	54.3	54.6	53.9	149.7	117.9	100.8	67.8
October–December	5. Corn	106.7	109.1	106.1	109.9	119.8	130.0	119.7	110.0	118.0
1922, January–April	6. Miscellaneous	.....	57.4	64.4	68.1	120.0	287.2	621.7	415.6	844.6
April–June	7. Corn	53.7	67.2	50.4	50.8	55.9	64.2	87.8	70.7	74.2
1920–22	(4) Corn (oats) crops	272.7	273.3	251.8	252.1	271.5	447.6	395.3	345.4	306.7
	(3) Miscellaneous crops	.....	142.6	142.1	152.7	330.3	1,565.8	1,108.6	1,499.5	1,696.8
	Total	272.7	415.9	393.9	404.8	601.8	2,013.4	1,497.9	1,844.9	2,003.5
1923, November–January	1. Corn	151.6	145.9	145.9	150.5	149.3	152.8	153.9	155.0	149.3
1924, February–May	2. Miscellaneous	.....	42.5	46.7	84.7	84.0	1,394.4	1,494.5	1,086.8	346.3
June–July	3. Corn	52.1	44.8	51.2	50.0	51.1	110.6	181.3	95.1	57.4
September–October	4. Corn	71.2	68.8	67.8	77.4	79.5	90.9	89.1	84.6	71.1
November–February	5. Miscellaneous	.....	30.2	38.2	35.6	41.8	260.8	220.5	247.0	110.0
1925, March–April	6. Corn	48.4	34.8	35.2	35.7	42.3	100.8	74.7	70.4	42.1
May–June	7. Corn	27.2	31.8	27.3	31.2	31.5	36.1	35.3	32.4	29.9
1923–25	(5) Corn crops	350.5	326.1	327.4	344.8	353.7	491.2	534.3	437.5	349.8
	(2) Miscellaneous crops	.....	72.7	84.9	120.3	125.8	1,655.2	1,715.0	1,333.8	456.3
	Total	350.5	398.8	412.3	465.1	479.5	2,146.4	2,249.3	1,771.3	806.1

Rich soil

1923, November–January	1. Corn	195.8	185.0	189.0	183.6	186.3	185.0	182.2	182.2	182.2
1924, February–May	2. Miscellaneous	.....	78.4	81.2	89.7	128.0	921.0	966.7	781.9	103.7
June-July	3. Corn	120.0	78.5	76.0	72.0	77.0	204.0	239.0	164.3	95.5
September–October	4. Corn	80.4	74.5	74.6	81.4	73.2	92.0	99.6	87.7	66.1
November–February	5. Miscellaneous	.....	42.8	43.6	48.0	33.0	257.3	189.0	215.6	71.2
1925, March–April	6. Corn	78.3	53.4	55.3	57.0	84.2	133.6	103.8	99.0	56.4
May–June	7. Corn	40.0	40.8	41.1	42.6	39.8	46.6	49.6	44.7	41.3
1923–25	(5) Corn crops	514.5	432.2	436.0	436.6	460.5	661.2	674.2	577.9	441.5
	(2) Miscellaneous crops	.....	121.2	124.8	137.7	161.0	1,178.3	1,155.7	997.5	174.9
	Total	514.5	553.4	560.8	574.3	621.5	1,839.5	1,829.9	1,575.4	616.4

To eliminate any trace of the previous differential treatments, all pots were emptied, their contents thoroughly mixed, and one or two test crops grown. The first set of rotation experiments was made on only the poor soil from September, 1920 to June, 1922. A second set was run simultaneously on both soils from November, 1923 to June, 1925.

In every case four or five evenly distributed rows of eight pots were planted in regular alternation to corn (oats, in one case) and to miscellaneous crops. The latter comprised four cereals (wheat, rye, oats, and corn) and four legumes (Canada field pea, hairy vetch, cowpea, and soybean). In addition there were five rows not planted when the miscellaneous crops were grown, but corn was raised on them as on all other rows.

Table 4 shows the average dry weights of the different crops, and table 5 the nitrogen contained therein.

These rotations were started with corn. The very uniform data obtained show the accuracy of these average figures, which are based on 32 single determinations (in the no-crop series, on 40). Seasonal influences, however, cause wide fluctuations, but if crops grown at the same season in different years are compared, remarkable uniformity is noticeable. This may be seen from the data for corn grown on the poor soil in May to July, 1921 and in June and July, 1924.

A comparison of the total figures for dry weights and for nitrogen harvested in the seven crops shows in every case that all cereal rows, with the exception of the dry weight for the corn row in the first test, were far below those recorded for the legume rows. The latter were generally two to three times higher in dry weight, and three to five times higher in nitrogen, with the exception of the soybean rows in the last two tests. If no crops were grown between the corn crops, the result was always below that of the rows planted continuously to corn. The most interesting fact is that the total crops in cereals were not lower—in the last two tests they were even distinctly higher—than in the permanent cereal rows. Four or five corn crops grown under the influence of legumes were equal or superior to seven corn and small grain crops grown without legumes.

The two crops of legumes on the poor soil in 1924 were about equal to the three crops raised on the same soil two years earlier, and they were markedly higher in dry weight and nitrogen. This originally rich soil which, 10 years earlier, produced much heavier crops than the poor soil, has now, after 23 crops, almost reached the same low level of productivity. The better growth of legumes on the poor soil will probably soon establish complete uniformity for both soils under this system of crop succession. Already the characteristic differences in the behavior of the four legumes tested are very marked and uniform on both soils. Field peas exert the most beneficial effect, followed by vetch and cowpeas, whereas soybeans are regularly more or less inferior. It must be emphasized in this connection, that the first three plants are usually still green when cut, whereas the soybeans are ripe. In the first test the field peas also (January to April, 1922) produced ripe seed, and there was, indeed,

TABLE 6

Influence of time upon after-effect to corn

INTERVAL	RICH SOIL*						INTERVAL	RICH SOIL†						INTERVAL	POOR SOIL†					
	Dry weights			Nitrogen				Dry weights			Nitrogen				Dry weights			Nitrogen		
	After no crop	After pea	+ −	After no crop	After pea	+ −		After corn	After pea	+ −	After corn	After pea	+ −		After corn	After pea	+ −	After corn	After pea	+ −
weeks	*gm.*	*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*weeks*	*gm.*	*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*weeks*	*gm.*	*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*
0	11.8	11.8	0	113.0	118.0	5.0	0	11.2	12.4	1.2	80.6	86.8	6.2	0	9.7	11.8	2.1	69.9	82.6	12.7
1	16.9	18.0	1.1	136.0	144.9	8.9	2	13.1	17.4	4.3	86.5	114.8	28.3	2	10.4	12.6	2.2	60.3	85.7	25.4
2	18.1	20.1	2.0	162.4	172.8	10.4	4	14.9	17.9	3.0	93.9	114.6	20.7	4	10.5	12.5	2.0	67.2	80.0	12.8
3	22.1	24.0	1.9	172.4	188.2	15.8	6	19.2	23.6	4.4	119.0	146.3	27.3	6	11.9	14.4	2.5	83.3	86.4	3.1
4	23.1	25.9	2.8	174.6	190.9	16.3	8	21.6	34.8	13.2	138.2	208.8	70.6	8	15.0	19.6	4.6	78.0	105.8	27.8
5	23.5	26.3	3.8	205.2	224 6	19.4	10	38.1	50.0	11.9	221.0	310.0	89.0	10	21.8	28.1	6.3	104.6	157.4	52.8
6	27.0	31.4	4.4	216.0	242.3	26.3	12	26.6	33.3	6.7	170.2	213.1	42.9	12	14.8	18.3	3.5	94.7	120.8	26.1
7	30.5	34.2	3.7	225.7	257.9	32.2	14	26.4	30.4	4.0	184.8	237.1	52.3	14	16.4	19.8	3.2	118.1	142.6	24 5
8	33.0	38.5	5.5	236.0	274.6	38.6	16	21.8	26.5	4.7	222.4	238.5	16.1	16	14.0	17.7	3.7	123.2	141.6	18.4
9	37.6	45.5	7.9	240.2	297.0	56.8	..	....	....	....	.....	.....	....	..	....	....	...	.....	.....	....
Average.........			3.3	.....	.....	23.0	..	....	....	6.0	.....	.....	39.3	..	....	....	3.3	.....	.....	22.6

* Corn planted April 1 to June 9, harvested June 7 to August 10, 1922.

† Corn planted April 26 to August 30, harvested June 28 to November 1, 1923.

very little increase in the following corn crop. It is true that the total nitrogen in this pea crop was unusually low, but this does not fully explain the situation. The total nitrogen harvested in the various leguminous crops frequently has no relation to the effect upon the next corn crop. Cowpeas, for instance, gave higher nitrogen returns than vetch during September to November, 1920, February to May, 1921, and November to February, 1924, on both soils, but the lower corn crops always came after cowpeas. During January to April, 1922, the nitrogen in soybeans was much higher than in vetch, but again the soybeans showed the lower after-effect.

When two corn crops were grown after legumes the after-effect was almost negligible in the second crop, but there was likewise a low effect upon the first corn crop noticeable if this was planted very soon after the legumes were harvested. This was the case with the last corn crop grown in the first test (April to June, 1922). As a rule the pots were replanted about four weeks after the previous crops had been cut, but in this case the corn was sown within a week. Some special tests in this direction seemed necessary.

After thorough mixing and preliminary testing of the soil, alternating rows of eight pots were planted throughout the house to corn and to peas, respectively, and the crops were removed. Immediately afterward, four rows evenly distributed through the house were replanted to corn, two after corn and two after peas. This was repeated at weekly intervals until after nine weeks all 36 rows were replanted. The harvesting was done in the same succession after two months. (See table 6.) There was no difference in dry weight and very little in nitrogen, immediately after the preceding crops were removed, but there was a gradual increase in the differences in dry weight and still more in nitrogen up to the end of the test. Accordingly, this experiment was repeated the next year on both soils, but this time two-week intervals were chosen in order to secure more complete data. (See table 6.) Again the differences were very small in the beginning, they reach their maximum after eight to ten weeks, and then they decline; but even after sixteen weeks they were considerably larger than at the beginning, and although they rise and fall generally with the crop weights at the different times of the year, there are still certain exceptions to this rule. This is especially noticeable with the total nitrogen returns at the end of the tests. After a temporary decline a second maximum is reached in the fall, which is evidently due to the increase in soil nitrification. The differences between the nitrogen returns also decline rapidly, and if the tests could have been continued, probably uniform results would soon have been obtained.

That the larger crops harvested after legumes are not due to an increased consumption of nitrogen originally present in the soil was indicated by the chemical analyses made at the end of the eight-year crop rotations in the field. The plots on which legumes were grown always showed the highest nitrogen percentage. The analyses of the soils in the pots furnish confirmative and more decisive data. The original nitrogen content in 1915 was 0.103 per cent

in the poor soil and 0.143 per cent in the rich soil. At the end of the second rotation in 1925, the following results for the differently treated rows were obtained (per cent N, average of four rows):

	NO CROP	WHEAT	RYE	OATS	CORN	PEA	VETCH	COWPEA	SOYBEAN
Poor soil	0.059	0.062	0.061	0.058	0.062	0.062	0.063	0.062	0.060
Average		Cereals: 0.061				Legumes: 0.062			
Rich soil	0.107	0.108	0.108	0.106	0.109	0.108	0.105	0.105	0.105
Average		Cereals: 0.108				Legumes: 0.106			

The small differences in the nitrogen content of cereal and legume rows are within the fluctuations always to be found in such soil tests. (A deviation of 0.001 per cent is equivalent to 90 mgm. nitrogen per pot—20 pounds soil.) Since both soils were uniformly mixed before the last rotation experiment was started, in order to eliminate any possible influence of the preceding experiments, such uniform results were to be expected. Marked differences, however, will become noticeable after these crop rotations will have been repeated several times. At present two facts stand out very clearly: (*a*) The original nitrogen content has been lowered to about three-fifths in the poor soil, and to three-fourths in the rich soil; (*b*) the soil under legumes is not lower in nitrogen, despite the larger amounts removed in the crops.

A complete nitrogen balance (per pot) for both soils presents the following picture, if it is based upont the data recorded for the crops grown on the (unmanured) check rows in the green manuring experiments, and on those raised in the permanent corn rows in the rotation experiments or in special tests as discussed before:

	NITROGEN REMOVED			
	From poor soil		From rich soil	
		mgm.		*mgm.*
Green manuring experiments	11 crops	810	8 crops	1,640
1. Rotation and special tests	11 crops	1,010	8 crops	1,250
2. Rotation experiments	7 crops	480	7 crops	620
Total	29 crops	2,300	23 crops	3,510
Original nitrogen in 20 pounds = 9000 grams soil	(0.103%)	9,270	(0.143%)	12,870
Added in water*		250		500
Green manure residues†	(50%)	150	(20%)	90
Total nitrogen available		9,670		13,460

	NITROGEN REMOVED			
	From poor soil		From rich soil	
		mgm.		*mgm.*
Soil nitrogen at end of experiment	(0.061%)	5,490	(0.107)	9,630
Reduction in soil nitrogen		4,180		3,830
Removed in crops		2,300		3,510
Difference		1,880		320
Equivalent to a loss in original nitrogen of		20%		2.4%

* Since the water sprayed on the pots has not been measured, the nitrogen added in it has been calculated from its analysis (0.4 p.p.m. total nitrogen) and from the high estimate that 500 parts water were used for the production of 1 part dry matter. The 29 crops on poor soil contained 1010 gm., the 23 crops on rich soil, 2116 gm. dry matter.

† The green manures applied averaged 300 mgm. N per pot. Since only 50 per cent of it was taken up by the manured crops on poor soils and 80 per cent on rich soil, and later in mixing the soil the residues were evenly distributed in all pots, the remaining nitrogen had to be added to the original soil nitrogen.

The small difference found for the rich soil is insignificant (an analytical deviation of 0.004 per cent nitrogen is equivalent to 360 mgm. nitrogen per pot), but there is no doubt that large losses have occurred in the poor soil. The bad physical character of the heavy clay favors denitrification, which probably has also been mainly responsible for the imperfect action of the green manure nitrogen in this soil.

A comparison of the nitrogen harvested on the poor soil in the permanent corn experiments and in those wherein corn alternated with peas, presents the following picture:

	NITROGEN REMOVED	
	By corn after corn	By corn and peas
	mgm.	*mgm.*
1. Rotation and special tests	1,010	2,420
2. Rotation	480	2,150
Total	1,490	4,570
More nitrogen in the corn-pea rotations	(18 crops)	3,080

Compared with the loss of 1880 mgm. nitrogen in the permanent corn experiment, a gain of 1200 mgm. nitrogen is to be recorded for the corn-pea series. Accordingly, an average gain in nitrogen from the air was obtained by the growth of one crop of peas equivalent to 600 mgm. per pot or approximately 200 pounds per acre (calculated as 3 million pounds of soil). The excellent

growth of the peas under greenhouse conditions is the cause of these high figures, which indicate the possibilities offered by a frequent return of legumes in crop rotations, although these possibilities can not be fully realized in the field.

Laboratory experiments

During the last two years laboratory experiments were made regularly and in a fairly comprehensive manner in conjunction with the last crop rotation experiments on both soils, and to some extent with the field experiments.

Thoroughly mixed soil samples taken at frequent intervals from differently treated pots or plots were used for the following determinations:

(*a*) Total counts of colonies grown on soil extract agar.
(*b*) Counts of colonies of the Radiobacter group on nitrate glycerin agar.
(*c*) Counts of colonies of Actinomycetes on the same agar.
(*d*) Counts of mold colonies on acid nitrate dextrose agar.
(*e*) Nitrates produced in 20 days in 50 cc. 0.1 per cent ammonium phosphate solution inoculated with 5 gm. soil.
(*f*) Nitrogen assimilated from the air in 10 days in 100 cc. 1 per cent mannite solution inoculated with 10 gm. soil.
(*g*) Amount of nitrates present in the soil samples.

Details about the methods used have been published elsewhere (23*a*, 32). The acid nitrate dextrose agar was prepared after the following formula: 1000 cc. soil extract, 1 gm. KH_2PO_4, 1 gm. KNO_3, 10 gm. dextrose, 30 gm. agar, adjusted to pH 4.0 to 4.5 by mixture of hydrochloric and sulfuric acid.

The nitrates present in the soil were determined by the phenol-disulfonic-acid method. The extract of 100 gm. soil was clarified by adding 50 cc. of a suspension of Al $(OH)_3$ before filtering.

The results presented in tables 7 to 9 are based upon the data obtained with four composite samples of the differently treated soils (two from poor, two from rich soil), each of them prepared from the samples taken in about 4 inches depth from the eight pots of every row. Four plates were poured in every case; the figures given for each soil are, therefore, the average of eight single determinations. The great accuracy of the averages based upon these figures is clearly demonstrated by the uniform figures obtained for the total counts of bacteria, of actinomycetes, and of fungi, at the beginning and at the end of the experiment, respectively.

On account of the conspicuous fluctuations in the total bacterial counts the results recorded in table 7 are given separately for every sampling, whereas in tables 8 and 9 merely average results have been inserted. As not all differently planted rows could be sampled simultaneously, the most important crops have been selected for these examinations.

The total bacterial counts under the different crops as given in table 7 and shown graphically in figure 1, leave no doubt about the very pronounced influence exerted by most of the legumes upon the soil microflora. The curves

TABLE 7

Number of colonies on soil extract agar

Millions per gram soil

(P = poor soil, R = rich soil, A = average count)

SAMPLING	NO CROPS			CORN			WHEAT			SOYBEAN (1924) VETCH (1925)			COWPEA			FIELD PEA (1925)		
	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A
February 4, 1924	68	38	53	64	38	51	61	43	52	68	40	54	70	37	51	...	...	...
February 25, 1924	47	60	54	62	43	53	53	64	58	55	47	51	51	73	62	...	...	...
Before planting	58	49	54	63	41	52	57	54	55	62	44	53	60	55	57	...	...	...
March 17, 1924	39	58	48	70	78	74	59	62	59	53	55	54	52	67	60	...	...	...
April 1, 1924	58	39	49	86	67	76	82	50	66	63	44	54	68	53	60	...	...	...
April 28, 1924	44	36	40	66	59	62	57	49	53	45	45	45	59	55	57	...	...	...
May 14, 1924	56	48	52	68	53	60	67	43	55	52	47	50	60	55	57	...	...	...
Under various crops	49	45	47	72	64	68	66	51	58	53	48	51	60	58	59	...	...	...
June 16, 1924	57	52	55	54	64	59	68	70	69	58	56	57	99	188	144	...	...	...
July 22, 1924	146	30	88	65	51	58	62	56	59	81	60	71	100	110	105	...	...	...
Under corn 1	101	41	71	60	58	59	65	63	64	70	58	64	100	147	124	...	...	...
October 20, corn 2	38	25	32	52	37	45	61	38	50	74	40	57	74	47	60	...	...	...
Averages, 1924	61	45	53	65	55	60	63	53	58	61	49	55	70	76	73	...	...	...

December 8, 1924	57	18	38	88	51	70	88	51	70	127	66	97	117	76	97	133	119	126
December 20, 1924	54	30	42	79	63	71	86	48	67	121	66	94	65	59	62	152	122	137
January 12, 1925	49	37	43	45	32	39	54	50	52	81	60	71	81	46	64	95	78	87
January 26, 1925	47	27	37	55	46	51	56	36	46	83	48	66	63	47	55	165	116	145
Under various crops	52	28	40	67	48	58	71	46	59	103	60	82	81	57	69	136	109	123
March 23, 1925	40	22	31	64	42	53	57	64	61	76	60	68	86	60	73	97	93	95
April 20, 1925	42	26	34	56	42	51	56	45	51	90	51	71	96	61	79	73	57	65
Under corn 1	41	24	33	60	42	52	57	55	56	83	56	70	91	61	76	85	75	80
June 6, corn 2	60	52	56	76	55	66	76	53	65	108	57	83	99	83	91	115	61	88
August 3, bare	38	28	33	34	25	30	32	31	32	40	30	35	38	27	33	49	35	42
Averages, 1925	48	30	39	62	45	54	63	45	54	91	55	73	80	58	69	110	85	98

TABLE 8

Number of colonies of the radiobacter group and of actinomycetes, grown on glycerin nitrate agar, and colonies of fungi, grown on acid dextrose nitrate agar

Actinomycetes, millions per gram soil

Radiobacter group and fungi, 10,000 per gram soil

SAMPLING	NO CROP			CORN			WHEAT			SOYBEAN (1924) VETCH (1925)			COWPEA			FIELD PEA (1925)		
	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A
Radiobacter group																		
Before planting	50	50	50	120	70	95	100	44	72	90	60	75	100	65	83	...	...	...
Under various crops	20	10	15	80	40	60	70	50	60	50	50	50	111	111	111	...	...	...
Corn 1, 1924	30	10	20	50	40	45	50	50	50	70	40	55	240	260	250	...	...	...
Corn 2, 1924	30	0	15	40	40	40	60	20	40	70	30	50	80	60	70	...	...	...
Under various crops	12	1	7	61	65	63	59	35	47	95	42	69	114	77	95	158	253	206
Corn 1, 1925	38	3	21	69	54	61	83	103	93	124	76	100	171	84	122	173	133	153
Corn 2, 1925	141	18	80	56	43	50	86	45	66	155	58	107	243	54	149	203	25	114
Bare, dry	8	5	7	30	0	15	15	5	10	30	13	22	28	8	18	30	8	19
Averages	35	15	25	70	50	60	66	47	57	66	46	56*	132	100	116	151	161	156
										102	50	76						
Actinomycetes																		
Before planting	7.0	6.8	6.9	7.5	6.3	6.9	7.6	6.0	6.8	8.3	6.9	7.6	7.9	7.1	7.5	....	...	....
Under various crops	8.8	5.2	7.0	10.9	6.5	8.7	10.6	7.0	8.8	9.2	6.6	7.9	9.4	6.8	8.1	....	...	....
Corn 1, 1924	8.4	5.6	7.0	8.9	5.7	7.3	9.1	5.3	7.2	10.5	5.7	8.1	12.2	5.5	8.8	....	...	....
Corn 2, 1924	10.9	5.2	8.0	11.1	4.9	8.0	11.5	5.5	8.5	12.5	5.6	9.0	14.3	6.9	10.6	....	...	....
Under various crops	9.0	4.2	6.6	11.0	5.2	8.0	11.3	4.4	7.8	13.5	5.1	9.2	12.3	4.9	8.6	15.1	5.8	10.5
Corn 1, 1925	8.7	3.8	6.6	10.2	5.3	7.7	10.7	3.6	7.3	12.5	4.9	8.7	14.1	4.4	9.3	13.4	4.9	9.1
Corn 2, 1925	12.3	3.9	8.1	11.3	4.1	7.7	12.3	4.7	8.5	12.7	4.8	8.8	11.3	4.1	7.7	13.1	6.0	9.6
Bare, dry	9.4	5.4	7.4	9.7	4.9	7.3	8.3	5.6	7.6	10.2	6.8	8.5	10.9	6.4	8.7	10.8	6.7	8.8
Averages	9.0	5.0	7.0	11.2	5.6	8.4	10.2	5.4	7.8	9.5	6.3	7.9*	11.2	6.4	8.8	13.9	5.7	9.8
										14.0	5.2	9.6						

Fungi

Before planting	14	19	17	24	19	22	22	17	19	24	17	20	27	17	22	..	..	..
Under various crops	11	21	16	18	18	18	17	17	17	20	20	20	20	20	20	..	..	..
Corn 1, 1924	14	21	17	21	25	23	19	21	20	21	20	21	26	19	23	..	..	..
Corn 2, 1924	10	13	12	25	22	24	33	28	31	32	38	35	31	34	33	..	..	..
Under various crops	9	10	10	24	22	23	30	22	26	31	33	32	30	28	29	34	30	32
Corn 1, 1925	8	10	9	27	29	28	40	30	35	43	45	44	48	27	38	42	40	41
Corn 2, 1925	38	30	34	28	20	24	35	22	29	45	30	38	33	56	45	35	36	36
Bare, dry	9	28	19	8	23	16	10	21	16	7	27	17	10	33	22	12	28	20
Averages	12	17	15	22	21	22	25	21	23	23	21	22*	29	27	28	33	33	33
										32	35	34						

* 1924 soybeans, 1925 vetch.

TABLE 9

Azotobacter tests (mgm. N assimilated in 10 days in 1 per cent mannite solution), nitrification tests (mgm. nitrified in 20 days in 0.1 per cent ammonium phosphate solution), and nitrate determinations in soils (nitrate nitrogen, parts per million)

SAMPLING	NO CROP			CORN			WHEAT			SOYBEAN (1924) VETCH (1925)			COWPEA			FIELD PEA (1925)		
	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A
Milligrams N assimilated																		
Before planting	8.1	9.3	8.7	9.0	9.1	9.1	8.6	9.1	8.9	8.7	8.9	8.8	9.0	9.1	9.1	....	....	....
Under various crops	8.0	9.0	8.5	8.7	9.6	9.2	8.0	9.4	8.7	8.0	9.9	9.0	8.0	9.5	8.8	....	....	....
Corn 1, 1924	9.0	9.0	9.0	8.7	8.8	8.8	8.9	9.1	9.0	9.2	8.4	8.8	8.9	7.4	8.2	....	....	....
Corn 2, 1924	9.2	11.7	10.5	9.8	10.7	10.2	9.1	8.4	8.8	9.6	9.4	9.5	9.4	8.5	9.0	....	....	....
Under various crops	8.2	10.5	9.4	8.8	11.0	9.9	9.4	11.3	10.3	9.6	10.4	10.0	9.0	9.6	9.3	9.1	9.9	9.5
Corn 1, 1925	9.5	9.0	9.3	9.2	10.6	10.0	10.4	10.3	10.4	11.0	10.8	10.9	9.6	8.8	9.2	9.6	9.1	9.4
Corn 2, 1925	10.6	9.6	10.1	11.4	10.4	10.9	11.6	11.5	11.6	12.4	8.2	10.3	12.8	9.2	11.0	11.4	11.6	11.5
Bare, dry	8.9	9.2	9.1	8.9	9.1	9.0	9.5	9.6	9.6	9.8	9.4	9.6	10.6	9.4	10.0	9.5	8.8	9.2
Averages	8.6	9.5	9.1	9.0	9.8	9.4	9.1	10.0	9.6	8.6	9.3	9.0	9.2	9.2	9.2	9.6	9.8	9.7
										10.3	10.1	10.2						
Milligrams N nitrified																		
Before planting	6.9	5.4	6.2	7.0	6.0	6.5	6.6	5.8	6.2	6.7	5.7	6.2	5.7	5.5	5.6	...	...	...
Under various crops	7.0	5.6	6.3	6.6	5.7	6.2	6.5	5.5	6.0	6.8	5.5	6.2	7.0	5.6	6.3	...	...	...
Corn 1, 1924	6.5	5.7	6.1	6.4	4.5	5.4	6.5	4.3	5.4	6.7	5.1	5.9	7.2	5.5	6.4	...	...	...
Corn 2, 1924	5.1	5.4	5.2	2.9	4.7	3.8	4.8	4.8	4.8	4.4	4.3	4.4	5.7	5.1	5.4	...	...	...
Under various crops	6.7	5.6	6.1	6.5	3.5	5.0	6.3	3.7	5.0	7.1	4.2	5.7	7.5	4.6	6.0	7.8	5.4	6.6
Corn 1, 1925	5.1	4.7	4.9	6.2	3.2	4.7	6.2	2.9	4.6	6.6	4.0	5.3	6.9	4.4	5.7	7.1	4.6	5.9
Corn 2, 1925	6.0	5.7	5.9	5.8	6.2	6.0	5.7	6.1	5.9	6.2	6.9	6.6	6.0	6.5	6.3	6.1	6.7	6.4
Bare, dry	6.6	5.4	6.0	5.8	5.2	5.5	5.7	5.1	5.4	6.3	5.4	5.9	6.5	4.9	5.7	7.0	5.5	6.3
Averages	6.4	5.4	5.9	6.2	4.6	5.4	6.2	4.6	5.4	6.5	5.2	5.9	6.8	5.1	6.0	7.0	5.5	6.3
										6.8	4.5	5.7						

Nitrate N in soils

Under various crops	11.4	11.6	11.5	3.0	3.8	3.4	2.9	2.4	2.7	4.0	3.6	3.8	1.6	2.6	2.1	5.6	7.9	6.8
Corn 1, 1925	13.3	22.3	17.8*	1.3	2.7	2.1	1.1	1.4	1.3	2.5	3.3	2.9	1.3	2.8	2.1	3.4	3.9	3.7
Corn 2, 1925	1.1	1.2	1.2	2.1	1.3	1.7	2.0	1.6	1.8	3.8	1.5	2.7	3.2	2.0	2.6	3.0	1.5	2.2
Bare, dry	5.2	9.4	7.3	7.8	12.8	10.3	7.0	12.0	9.5	7.4	14.5	11.0	7.1	11.7	9.4	7.1	13.5	10.3
Averages	12.3	17.2	14.8*	3.1	4.5	3.8	2.8	3.6	3.2	4.0	5.0	4.5	2.7	4.1	3.4	4.7	6.4	5.6
	3.1	5.3	4.2															

* The first two nitrate determinations were made with soil which was kept bare permanently. The last two determinations, however, were made with soil that was planted to corn after it had remained bare while miscellaneous crops were grown on the other pails.

for field pea, vetch, and cowpea stand out conspicuously, whereas the results obtained under and after soybeans are not very different from those found with cereals. In their effect upon the succeeding crops the legumes tested ranked as follows: peas, vetch, cowpea, soybean. The averages of the bacterial counts are for pea 98, vetch 73, cowpea 69, and soybean 55 millions, a very close coincidence. However, the wide fluctuations recorded for the various dates demonstrate clearly that decisive results can not be expected unless such tests are made upon a broad basis and for long periods. In addition, a comparison of the numbers of bacteria, as well as of actinomycetes, found in both soils leaves no doubt that there exists no direct correlation between the number of soil bacteria and soil productivity. As in the present case, an increase in the number of soil organisms may accompany, or in part even cause an increase

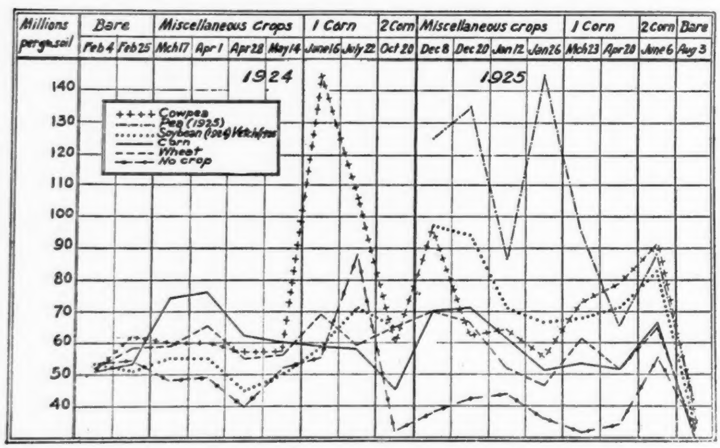

FIG. 1. TOTAL BACTERIAL COUNTS UNDER AND AFTER DIFFERENT CROPS GROWN IN POT EXPERIMENTS

in, soil productivity, but the fertility of different soils is dependent on so many other factors that no uniform correlations can be expected (23, p. 513).

Actinomycetes, as well as molds, were slightly more numerous under and after legumes than under cereals. Very wide differences occurred within the so-called Radiobater group. (Table 8.) These organisms play an important rôle in the soil, because of their ability to assimilate elementary nitrogen as well as nitrate nitrogen. They were much more numerous under and after field peas and cowpeas than under cereals, whereas they were almost absent in the no-crop series, despite the high nitrate content of these soils. (Table 9.) A special experiment was made in order to ascertain whether an increase in Radiobacter growth could be induced by adding straw to these soils rich in nitrate. With the poor soil, positive results were obtined, whereas in the rich soil a marked stimulation in mold growth took place. Since many members

of the last named group are also able to assimilate nitrate nitrogen very rapidly under suitable conditions, this result is not surprising. The different behavior of the two soils is explained by the fact that the poor soil is alkaline (pH 7.4 to 7.6), whereas the rich soil is slightly acid (pH 6.6 to 6.8).

At present it is impossible to decide definitely whether the activity of members of the Radiobacter group under and after legumes is greater in nitrate assimilation or in the fixation of elementary nitrogen. The last named activity, however, probably predominates. Table 9 shows that the soils contained more nitrate nitrogen under and after legumes (especially pea and

TABLE 10

Average results of bacteriological and biochemical tests of field soils

		FIELD I			ORIGINAL SOD BETWEEN FIELDS I/II	FIELD II	
		Corn	Cowpea	Soybean		Wheat	Vetch followed by potatoes
Bacterial counts, millions per gram soil	1924	24	27	26	21	27	....*
	1925	25	25	23	19	25	54
Radiobacter group 10,000 per gram soil	1924	35	40	35	25	30	....
	1925	34	42	33	15	24	45
Actinomycetes, millions per gram soil	1924	5.6	5.8	5.5	7.2	6.1	....
	1925	6.5	7.0	7.0	7.6	7.7	9.7
Fungi; 10,000 per gram soil	1924	9	14	11	10	16	....
	1925	11	13	11	12	14	17
Mgm. N assimilated in mannite solution	1924	7.6	6.7	6.7	2.9	3.3	....
	1925	6.3	6.0	5.6	3.7	3.2	4.4
Mgm. N nitrified in $(NH_4)_2HPO_4$ solution	1924	4.4	4.7	5.0	3.2	4.3	....
	1925	5.1	5.6	5.7	3.3	4.0	5.7
Nitrate N in soil, parts per million	1924	....*	....	....	....	....	....
	1925	3.0	11.0	7.9	0.52	1.20	13.6

* No determinations made.

vetch) than under cereals, this does not indicate a strong nitrate assimilation by soil bacteria.

The results of nitrification experiments in ammonium phosphate solution, recorded in table 9, indicate a stronger nitrification under and after legumes, confirming and supplementing the nitrate determinations made directly with the different soils. The nitrogen assimilation in mannite solution, on the other hand, has not proved to be superior under legumes. It must be kept in mind, however, that the conditions of this experiment are favorable to Azotobacter,

as well as to Amylobacter, but are not especially suited to the requirements of other nitrogen-fixing bacteria, such as *B. radiobacter*. Therefore, the only conclusion justified is that Azotobacter and Amylobacter were no more stimulated in their development by the growth of legumes than by that of non-legumes.

Alternating with the experiments made with greenhouse soil, analogous tests were made with samples taken from part of the field plots during the growing season. In table 10 the averages are given as calculated from 4 and 5 successive tests, made from April to August, 1924 and from March to July, 1925, respectively. The consistency of the results in both years is quite marked, and it is likewise evident that the data obtained under field conditions agree well with those secured in the greenhouse. Since the soil of field I is somewhat different from that of field II, only the effects of the crops grown on the same field can be compared unrestrictedly. The much stronger nitrogen fixation in mannite solution inoculated with soil from field I is of special interest in view of its increased productivity discussed before.

Under and after legumes again the total bacterial counts are somewhat larger than under cereals and the number for the Radiobacter group is markedly higher under vetch and cowpeas, but not under soybeans. The actinomycetes and fungi show slight changes again in favor of the legumes, as do the nitrification and to a still larger extent the nitrate content of the soil.

Late in the fall of 1924 the total number of bacteria under cowpeas reached a maximum of 47.5 million, and the Radiobacter group 1,400,000 colonies per gram soil. At the same time the plowed wheat stubble showed a total count of 44.5 millions, and 470,000 Radiobacter colonies per gram soil. The tilth of the soil was very satisfactory at this time.

The bacterial counts obtained with samples taken from a strip of original sod that was left untouched between fields I and II when the field experiments were started in 1914, show how uniform the results of such tests will be if the environmental conditions are uniform and a faultless technique is carefully applied. Throughout the year 1924, the following total counts (millions per gram soil) were secured on soil extract agar, as the average of two soil samples taken under the sod 4 inches below the surface: April, 21.8; May, 19.0; July, 20.0; August, 22.8; October, 20.1; November (2 tests), 21.8 and 17.0.

These widely fluctuating results, which are not unusual (32), should not be ascribed to erratic daily or hourly variations in the bacterial flora of the soil.

DISCUSSION

The experiments reported have shown conclusively that the beneficial after-effect exerted by legumes harvested for hay is to a considerable extent due to favorable changes in the microflora of the soil, which are still marked, and are even increasing a few weeks after the surface growth of the legumes has been removed. Several months later, and especially after the soil has dried out thoroughly, this beneficial effect upon the succeeding crop is no longer

noticeable. The increases in the succeeding crops caused by this after-effect of harvested legumes are frequently larger than those caused by legumes used as green manures. It is probable that in field tests the crop increases ascribed to green manuring are in fact more frequently due to the special after-effect of the growing legumes than to their manuring effect after having been plowed under where they had been grown.

These findings are supported by practical experience as well as by earlier experimental results. Tests made at the Arkansas Station more than twenty years ago (29, 30, 31) showed that the increases in the winter wheat crop were larger when the legumes were removed than when they were turned under, but that with spring oats, opposite results were obtained. Analogous findings were recorded at the same time in Alabama (6) where they were accepted as "unexpected." Again winter wheat and winter oats were more benefited by the legume stubble than by the legume as green manure, whereas with the crops planted in spring (cotton, corn, sorghum) opposite results were obtained. In a cowpea-wheat rotation tested at the Tennessee Station (26) practically the same wheat crop was raised whether the surface growth of the cowpeas was removed or whether it was turned under. Experiments made in Virginia (1) with clover, soybeans, buckwheat, and rye on corn gave likewise approximately the same results with the stubble as with the whole plant turned under. In cylinder experiments conducted at the New Jersey Station (21) the nitrogen returns were not much higher if cowpeas, crimson clover, or winter vetch were turned under where they had been grown, than in those cases where their surface growth was removed and tested separately.

Field peas and vetches were found by J. G. Lipman (20) to exert a more beneficial effect upon non-legumes than cowpeas and soybeans, and the same relation was noticeable in our experiments. Practical experience (27) as well as the pot tests reported, shows that soybeans are regularly less beneficial to the succeeding crop than are cowpeas. The fact that not more than two per cent of the legumes grown in America are being used as green manures furnishes additional proof that it is the beneficial after-effect of the growing legumes which is of greatest importance.

The detrimental effect sometimes noticed with heavy green manuring, but more frequently overlooked because of the counter-action due to the beneficial after-effect of the growing legumes, may have several causes. If relatively large quantities of green matter are used, as is often the case in pot tests (36), acid and ammonia production, heavy mold growth, and other factors which are hardly of any importance under field conditions, may prove detrimental. Nitrate assimilation is probably of greatest importance in the field, since nitrification is enhanced under and after legumes, whereas the addition of fresh organic matter tends to stimulate the opposite reaction. To use leguminous green manure on a field other than where it was grown will give better results, as a rule. Mulching deserves special attention in this connection (12).

Generally preferable, however, is the feeding of the legumes and the application of the stable manure obtained from them, as is demonstrated by the

increased crops and the unimpaired nitrogen content of the plots so treated in our field experiments. It is true that the humus and nitrogen content of a soil may remain constant for some time, even if only the crop residues are being left (2). As a rule, and especially after the original humus content of a soil is as far reduced as is the case in many fields of the eastern and southern part of the United States, the use of organic manures is essential to restore and maintain the vanishing natural soil productivity. A comparatively small amount of stable manure, however, is sufficient for this purpose, and as shown by the field experiments an unusually high efficiency may be secured in this case.

The low nitrogen efficiency of mineral nitrogenous fertilizer recorded in the second half of the crop rotation experiment in the field is undoubtedly due to lack of humus, causing a deterioration of the soil texture and an insufficient production of carbon dioxide. Equally incomplete nitrogen returns have been noted in other tests made with nitrate under similar conditions (22). As in the field experiments discussed, the after-effect of legumes cut for hay was equal or superior to the application of nitrate, whereas ripe legumes again proved inferior (11).

It has been stated occasionally that "very revolutionary practices" would have to be instituted in this country in order to retain proper relations between food supply and increasing population (8). The experiments discussed, however, show that it is quite feasible to double and treble the grain crops within a few years by growing cereals and legumes best adapted to soil and climate in suitable rotations, and there is no doubt that at least in all regions where legumes can be grown successfully, their proper use will assure much larger grain crops than are needed at the present and in the near future. Wherever the natural soil productivity is now rather low, because of a long continued growth of grain crops, it seems highly advisable from an economic standpoint to raise the same quantities of cereals on a smaller tilled area by adopting more efficient crop rotations, and to increase at the same time the natural fertility of the remaining area by using it for pasturage or for producing forage rich in legumes. Any future emergency would then easily be overcome by returning this enriched land to the production of heavier grain crops.

SUMMARY

Field, greenhouse, and laboratory experiments on the beneficial influence exerted by growing legumes upon succeeding crops have furnished the following results:

1. Comparative field experiments showed that the increases in the crops after legumes were almost or quite as high when the legumes were harvested as when they were plowed under as green manures. The maximum increase observed in pot tests was close to 100 per cent by green manuring, but over 200 per cent by the growth of legumes harvested for hay.

Several reasons of this beneficial after-effect were discussed and investigated. In the field the suppression of noxious weeds is of great value, whereas not much importance can be attached to the amount of nitrogen contained in stubble and roots. Probably of greatest importance is the stimulating effect exerted by the growing legumes upon the bacterial activities in the soil.

2. The stimulation of bacterial activities by growing legumes was evidenced by marked increases in the total number of soil organisms enumerated on soil extract agar, by rapid multiplication of *B. radiobacter* and related forms, and by greatly intensified nitrification, Actinomycetes and fungi became likewise more numerous under the influence of legumes. though to a much smaller extent than the bacteria. Numbers of *B. radiobacter* as high as under legumes were observed only once in a plowed wheat stubble after this soil had attained its perfect tilth in fall. The favorable change in the soil flora persisted and became even more pronounced during the next two or three months after the legumes had been harvested. Longer intervals, however, and especially a thorough drying of the soil caused a more or less complete disappearance of these beneficial changes.

3. These observations explain the great benefit to the soil productivity of replanting soon with cereals or hoed crops a field upon which legumes have been grown. Naturally, a marked after-effect is dependent on the proper selection of the legumes used in the crop rotation; these will be different according to soil and climate. On the experimental field only three of a large number of legumes proved entirely satisfactory, viz., cowpeas and soybeans as summer crops, and hairy vetch as a winter cover crop. These legumes, grown every fourth year on the field, assimilated from the air approximately 80 pounds nitrogen per acre and year and exerted, after their surface growth had been removed, an after-effect equivalent to that obtained by the application of 30 pounds nitrate nitrogen per acre and year.

4. Greenhouse tests showed that field peas, hairy vetch, and cowpeas were of greatest benefit to the microflora of the soil and to the succeeding grain crops. Soybeans, on the other hand, were hardly of any influence in this direction, although they gave quite satisfactory results when used as green manure. The growing of two or three crops of field peas, hairy vetch, or cowpeas for hay, alternating with five crops of corn increased the latter to such an extent that their total weight exceeded that of seven crops of corn and small grains raised without legumes.

5. In a heavy clay soil poor in humus, considerable losses of soil nitrogen occurred under 29 crops of non-legumes, whereas conspicuous gains in nitrogen, equivalent to one-third of the total nitrogen originally present in this soil, were secured when legumes were grown a few times between the non-legumes. No losses in nitrogen occurred in a soil of better chemical and physical quality. Despite greater natural productivity, this soil gave persistently lower counts in soil organisms than were obtained with the poorer soil.

6. A low humus content in field soil caused a rather low efficiency of nitrogen applied as nitrate or ammonium sulfate. Only 40 per cent was recovered in the crops. On the other hand, stable manure used in relatively small amounts (15 tons per acre in an eight-year rotation) showed a nitrogen efficiency of 75 per cent. Probably in addition to the improvement of the physical structure of the soil, the increased development of carbon dioxide from organic manures is of considerable importance under such conditions.

7. Long continued greenhouse tests on the effect of different crop successions are an excellent means of securing more definite and more thorough information upon this important subject. The precautions to be observed in such experiments have been discussed, and it has been shown that very reliable data can be secured, if these tests are made on a broad basis and for a sufficient length of time.

REFERENCES

(1) ANONYMOUS 1916–17 Report of the chemical department. *Va. Agr. Exp. Sta. Ann. Rept.*, 1917: 21, 22.

(2) BEAR, F. E., AND SALTER, R. M. 1916 The residual effects of fertilizers. W. Va. Agr. Exp. Sta. Bul. 160.

(3) BORDLEY, J. B. 1801 Essays and Notes on Husbandry and Rural Affairs, ed. 2. Philadelphia.

(4) BUFFUM, B. C. 1900 Alfaifa as a fertilizer. Wyo. Agr. Exp. Sta. Bul. 44.

(5) COTTRELL, H. M., OTIS, D. H., AND HANEY, J. B. 1901 Soybeans in Kansas. Kans. Agr. Exp. Sta. Bul. 100.

(6) DUGGAR, J. F. 1902 The cowpea and the velvet bean as fertilizers. Ala. Agr. Exp. Sta. Bul. 120.

(7) DYER, B. 1909–10 On Lucerne. With notes on some other leguminous crops. *New Zeal. Dairyman* 14: (3) 60–62, (4) 17–20.

(8) GEORGE, D. H. 1922 What is there left to do? *Sci. Amer.* 127: 382–383.

(9) GREAVES, J. E. 1918 Does crop rotation maintain the fertility of the soil? *Sci. Mo.* 6: 458–466.

(10) GROH 1925 Beitrag zur Frage der Ertragesteigerung durch den Anbau von Leguminosen. *Landw. Ztg.* 45 (15): 181–182.

(11) HALL, THOS. D. 1924 Legumes versus nitrate of lime as affecting the yields of barley. *Jour. Dept. Agr. So. Africa* 8: 505–507.

(12) HARLAN, C. 1912 Farming with Green Manures, ed. 7. Wilmington.

(13) HARTWELL, B. L., PEMBER, F. B., AND MERCKLE, G. E. 1919 The influence of crop plants on those which follow. II. R. I. Agr. Exp. Sta. Bul. 176.

(14) HASELHOFF, E. 1921 Gründüngung auf leichtem und schwerem Boden. *Fühling's Landw. Ztg.* 70: 407–418.

(15) HOLTZ, H. F., AND SINGLETON, H. P. 1925 Comparative value of alfalfa and sweet clover on soil in the lower Yakima valley. *Jour. Amer. Soc. Agron.* 17: 326–333.

(16) HOPKINS, C. G. 1913 The Illinois system of permanent fertility. Ill. Agr. Exp. Sta. Circ. 167.

(17) HOWARD, L. P. 1920 The reaction of the soil as influenced by the decomposition of green manures. *Soil Sci.* 9: 27–39.

(18) LAWES, J. B., AND GILBERT, J. H. 1895 The Rothamsted Experiments. Edinburgh and London.

(19) LECLAIR, C. A. 1915 Influence of growth of cowpeas upon some physical, chemical and biological properties of soil. *Jour. Agr. Res.* 5: 439–448.

(20) LIPMAN, J. G. 1912 The associative growth of legumes and non-legumes. N. J. Agr. Exp. Sta. Bul. 253.

(21) LIPMAN, J. G., BLAIR, A. W., OWEN, I. L., AND MCLEAN, H. C. 1912 Miscellaneous vegetation experiments. N. J. Agr. Exp. Sta. Bul. 250.

(22) LIPMAN, J. G., BLAIR, A. W., AND PRINCE, A. L. 1925 Field experiments on the availability of nitrogenous fertilizers, 1918–1922. *Soil Sci.* 19: 57–75.

(23) LÖHNIS, F. 1910 Handbuch der landwirtschaftlichen Bakteriologie, p. 578–582. Berlin.

(23*a*) LÖHNIS, F. 1913 Laboratory Methods in Agricultural Bacteriology. London.

(24) LYON, T. L., AND BIZZELL, J. A. 1913 Some relations of certain higher plants to the formation of nitrates in soils. N. Y. (Cornell) Agr. Exp. Sta. Mem. 1.

(25) LYON, T. L., BIZZELL, J. A., AND WILSON, B. D. 1920 The formation of nitrates in a soil following the growth of red clover and of timothy. *Soil Sci.* 9: 53–64.

(26) MOOERS, CH. A. 1911 Experiments with fertilizers and field crops, etc. *Tenn. Agr. Exp. Sta. Bul.* 92: 25–95.

(27) Mooers, Ch. A. 1924 The problem of forage crops in relation to soil improvements. *Jour. Amer. Soc. Agron.* 16: 236–238.

(28) Moore, Th. 1801 The Great Error of American Agriculture and Hints for Improvement Suggested. Baltimore.

(29) Newman, C. L. 1900 Wheat experiments. *Ark. Agr. Exp. Sta. Bul.* 62: 15–34.

(30) Newman, C. L. 1901 Cowpea experiments. Ark. Agr. Exp. Sta. Bul. 70.

(31) Newman, C. L. 1903 Cowpea experiments. Ark. Agr. Exp. Sta. Bull. 77.

(32) Smith, N. R., and Worden, S. 1925 Plate counts of soil microörganisms. *Jour. Agr. Res.* 31: 501–517.

(33) Stoklasa, J. 1924 Die modernen ziele der biochemischen Forschung des Bodens. *Chemie d. Zelle u. Gewebe* 12: 23–44.

(34) Swanson, C. O. 1917 The effect of prolonged growing of alfalfa on the nitrogen content of the soil. *Jour. Amer. Soc. Agron.* 9: 305–314.

(35) Swanson, C. O., and Latshaw, W. L. 1919 Effect of alfalfa on the fertility elements of the soil, etc. *Soil Sci.* 8: 1–39.

(36) Whittle, Ch. A. 1920 Legume nitrogen. South. Fert. Assoc., Soil Impr. Com. Bul. 33.

BOOK REVIEW[1]

Handbuch der biophysikalischen und biochemischen Durchforschung des Bodens. (*Handbook of Biophysical and Biochemical Soil Investigations.*) *1926.* STOKLASA, J. AND DOERELL, E. G. (Pp. xv + 812, fig. 91. Paul Parey, Berlin.)

The senior author of this book, Professor of the Technological Institute and State Experiment Station at Prague, Czechoslovakia, has produced, with the collaboration of the junior author, an exceptionally fine contribution to the subject of soil science. The senior author, who recently celebrated the fortieth anniversary of his scientific activities, himself took a prominent part in various phases of the development of our knowledge of microbiological processes in the soil and their bearing upon plant growth. The book will be of great value to all those who are interested in the soil as a medium for the growth of higher plants, and especially as a guide to the teacher and investigator. The authors succeeded in covering the literature of the subject quite thoroughly, largely avoiding the pitfalls of many other handbooks that frequently become merely an encyclopedic enumeration of literary contributions, without any critical interpretation.

The volume is made up of the following sections: Introductory. *Biophysical and biochemical investigations of the soil.* Methods of soil investigation. Mechanical soil analysis. Determination of water-holding capacity of the soil. Soil atmosphere. Chemical analysis of the soil. Adsorptive power of the soil. Determination of electrical conductivity of the soil. Soil reaction. Methods of determination of soil acidity. Gas-chain method of Michaelis. Field method of electrometric determination of soil acidity after M. Trénel. Colorimetric methods. Determination of hydrogen-ion concentration of soil by a modified method of Michaelis. Other methods for determination of soil reaction. Exchange acidity. Critical remarks concerning the soil reaction and its determination electrometrically and colorimetrically. Special chemical investigation of the soil. Determination of nutrients in soil extracts. Determination of radio-activity in soil and in soil air. On the influence of natural radio-activity of minerals and rocks upon the germination and development of plants. Methods of determination of radioactivity in soil and in soil air. *Methods of biological investigation of soil.* General considerations concerning the microörganisms present in the soil. The bacteria of the rhizosphere. Methods of investigation of the edaphon. Isolation of the edaphon population and the biochemical

[1] This review was prepared by Dr. Selman A. Waksman, of the New Jersey Agricultural Experiment Station.

characterization of individual geobiont groups. Bacteria that participate in the cycle of nitrogen in nature. Protein synthesis in the soil. Methane-decomposing bacteria. Hydrogen-oxidizing bacteria. Sulfur bacteria. Desulfurication in soil. Iron bacteria. Actinomyces. Fungi. Algae. Protozoa. Determination of excretory products in bacterial respiration. Biological absorption. Biochemical methods of determination of phosphoric acid and potash present in the soil in an assimilable form. Soil respiration. Carbon dioxide as an index of soil processes. Methods of determination of carbon dioxide evolution from soil. Experiments concerning the utilization of various organic substances by heterotrophic organisms as sources of carbon. Influence of the chemical nature of organic substances upon respiration processes in the soil. Decomposition of celluloses in the soil. Oxidation of organic nitrogenous compounds in the soil. Respiration intensity of microörganisms (auto- and heterotrophic) in various treated and untreated soils. Respiration of forest soils. Composition of drainage waters as an index of biochemical processes in the soil. On the influence of manure upon the mechanism of respiration in the soil. On the influence of radioactivity upon the dissimilation processes of microörganisms in the soils. Conclusions.

On the whole, the various phases of biochemical processes in the soil are extensively and adequately treated. The author emphasizes particularly the results of his own investigations during the long period of his scientific activities; however, other methods and results are considered sufficiently, so that the book has not been made too one-sided.

It is easy to find faults in a book of this nature, where practically virgin ground is covered and where an attempt is made to review a most extensive literature published in various languages, touching upon a number of phases of as complicated a subject as soil science. For example, one might say that altogether too much space is devoted to the determination of soil reaction (100 pages) and to the radioactivity of soil (46 pages) and that rather insufficient consideration is given to certain important groups of soil organisms—especially to those non-bacterial in nature; namely, the fungi, actinomyces and protozoa—and to certain microbiological activities, such as the nature of partial sterilization of soil. In speaking of soil adsorption and exchange of bases, the authors consider only the work of Hissink, without even mentioning the important contributions to this subject by Gedroiz. Although an excellent discussion is given of the methods of determination of "humus" and "humic acids," nothing is presented to indicate the origin of these organic complexes in the soil and the rôle of microörganisms in these processes. Too much emphasis is laid upon bacterial fertilizers, consisting of composts of peat and phosphoric acid inoculated with bacteria from the root system (rhizosphere) of cultivated plants; this process is even emphasized as a triumph of modern biochemical technic—unfortunately we have altogether too much evidence to indicate that the numerous "all-crop inoculants" have proved to be largely failures. The soil harbors sufficient organisms capable of pro-

ducing abundant transformations, if conditions are favorable to their development. If one is justified in inoculating a freshly drained and limed peat soil with good garden soil, or a normal field soil with certain specific organisms such as the nodule bacteria, the use of all-crop inoculants is more than questionable. One would also like to take exception to the statement (p. 396) that the use of Alinit was the stimulus to the development of the whole science of soil bacteriology—perhaps the discouragement experienced by many workers in the field is due to that kind of a stimulus. The reviewer does not want to give, however, too much consideration to the defects which tend to detract from the otherwise excellent qualities of the book. The literature is covered thoroughly; here again any disagreements that one might present would be rather in connection with a difference in interpretation of the results.

The book is well printed and has a most extensive bibliography, consisting of thousands of references, placed very conveniently at the bottom of the respective pages. Detailed author and subject indices are appended. Some misprints have been noted. Dr. H. J. Conn (p. 424) of the New York Experiment Station is referred to as the Swiss bacteriologist probably because of the fact that the Experiment Station is at Geneva, N. Y.

ON THE ORIGIN AND NATURE OF THE SOIL ORGANIC MATTER OR SOIL "HUMUS": IV. THE DECOMPOSITION OF THE VARIOUS INGREDIENTS OF STRAW AND OF ALFALFA MEAL BY MIXED AND PURE CULTURES OF MICROÖRGANISMS[1]

SELMAN A. WAKSMAN AND FLORENCE G. TENNEY

New Jersey Agricultural Experiment Stations

Received for publication March 6, 1926

It has been demonstrated that when straw is acted upon by pure cultures of fungi or bacteria in soil or in sand media, the sugars, hemicelluloses, and celluloses are readily decomposed, but the lignins are not acted upon. As a matter of fact the presence of the lignins even prevents, to a certain extent, the rapid decomposition of the celluloses with which they are combined. These lignins accumulate in the soil, especially in the absence of, or under conditions which do not favor the development of organisms capable of decomposing them. This accumulation contributes to the formation of the soil "humus." It remains to be seen whether a mixed soil flora will behave in normal soils in a similar way toward the lignins.

For this purpose, barley straw and alfalfa meal (green alfalfa, dried and ground) were treated in a manner outlined previously, for the purpose of removing consecutively different constituents or groups of the natural organic matter. Ether was used this time to remove the fats and waxes; this was followed by 95 per cent alcohol, then by cold water for 24 hours. The material from which these fractions were removed was then treated with 5 per cent NaOH solution for 30 minutes at 15 pounds pressure. The liquid was filtered off. The residue was washed with water and acetic acid and then treated with 2 per cent H_2SO_4 solution for 2 hours at boiling temperature. At every step of the process, portions were set aside and used afterward in the study of decomposition. Pure lignin was prepared for the following studies by a modification of the Willstätter method, suggested by Schwalbe (4). This consists in treating 3-gm. portions of the organic materials from which the ether, alcohol, and water-soluble portions have been removed, with a mixture of 72 per cent H_2SO_4 (60 cc.) and 15 cc. of 1:1 HCl (18 per cent), in a glass stoppered bottle, which is kept immersed in cold water during the reaction. After the reaction is completed, the mixture is transferred to a flask with about 500 cc. of distilled water and boiled for 1 hour. The lignin is then

[1] Paper No. 279 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

filtered through paper and washed until all traces of the inorganic acids have been removed.

An analysis of the two natural organic substances used is given in table 1.

One-gram portions of the different dry preparations were then added, in duplicates, to 100-gm. portions of sieved fresh soil, placed in 300-cc. round bottom flasks. Five-cubic centimeter portions of a nutrient solution containing 2 gm. $(NH_4)_2SO_4$ and 4 gm. K_2HPO_4 in 100 cc. of water, were added to each flask. The soil was then well mixed, brought to optimum moisture with distilled water and connected with the respirator used in the study of the evolution of CO_2; the whole apparatus was kept in the thermostat at 27 to 28°C. After 35 days incubation, in one case, and 32 days in another, the

TABLE 1

*Composition of barley straw and alfalfa meal**

PREPARATION	FRACTION NUMBER	STRAW		ALFALFA MEAL	
		Yield	Nitrogen extracted from 10 gm.	Yield	Nitrogen extracted from 10 gm.
		per cent	*mgm.*	*per cent*	*mgm.*
Original material, moisture content	I	9.38	....	12.30	.....
Ether-soluble substances	II	1.35	....	1.15	.....
Alcohol-soluble substances	III	3.14	....	4.64	.....
Loss on extraction with cold water for 24 hours	IV	7.46	8.8	15.41	33.0
Loss on extraction with 5 per cent NaOH at 15 pounds pressure	V	31.51	31.4	31.70	132.5
Precipitate of NaOH extract with HCl	VIII	19.06	....	10.98	.....
Lignin, by method of Schwalbe†	VII	15.58	....	14.67	.....
Loss on treatment with 2 per cent H_2SO_4	VI	10.90	....	8.96	.....
Residual material (cellulose) after treatment with 2 per cent H_2SO_4	VI	36.22	....	25.84	.....

* Total nitrogen content of the straw 0.41 per cent, of alfalfa meal 2.44 per cent.

† Determination made on preparation, after ether, alcohol, and water-soluble fractions have been removed, but calculated on basis of original moisture-free material.

soils were removed and aliquot portions used for the determination of ammonia (by replacement with KCl solution, then distilling with MgO), of nitrates (by the phenoldisulfonic acid method), and of "humus" (by the method reported above). The results are given in tables 2 and 3.

The removal of various fractions from natural organic materials, such as barley straw and alfalfa meal, by solvents, influences materially both the rate and the amount of decomposition of the organic matter, within a definite period of time. Although the separation of the various ingredients was probably in some cases incomplete, the results point definitely to the fact that natural organic matter contains ingredients which are acted upon more readily by the mixed soil flora and fauna, ingredients which are decomposed more

TABLE 2

*The decomposition of different fractions of barley straw by the mixed soil flora, in untreated soil**

PREPARATION	FRACTION NUMBER	CARBON EVOLVED AS CO_2 IN							TOTAL CO_2	CONTROL SOIL SUBTRACTED	RESIDUAL AVAILABLE NITROGEN $NH_3-N + NO_3-N$ IN 100 GM. OF SOIL	AMOUNT OF NITROGEN USED UP	HUMUS, α-FRACTION IN 100 GM. OF SOIL
		2 days	5 days	8 days	14 days	18 days	23 days	35 days					
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Control soil		5.8	4.8	3.4	6.0	5.8	5.2	13.8	44.8	.....	22.9	....	553
Untreated straw	I	17.7	29.1	21.5	36.9	22.0	14.0	27.4	168.6	123.8	16.0	6.9	673
Ether fraction removed	II	18.5	31.6	21.5	41.1	20.3	14.4	27.0	174.4	129.6	15.1	7.8	733
Ether and alcohol fractions removed	III	16.3	30.0	22.3	36.6	20.6	15.3	29.2	170.4	125.6	15.1	7.8	707
Ether, alcohol, and water-soluble fractions removed	IV	10.7	28.4	21.9	37.5	23.8	14.9	30.5	167.7	122.9	15.7	7.2	683
Ether, alcohol, water, and alkali-soluble fractions removed	V	11.4	33.9	30.1	43.0	30.9	20.0	35.8	205.1	160.3	13.5	9.4	610
Residual cellulose	VI	6.4	21.2	29.3	58.5	35.1	22.6	39.5	212.6	167.8	11.3	11.6	513
Alkaline extract precipitated with HCl	VIII	15.1	21.0	14.3	19.3	9.4	9.6	20.8	109.5	64.7	19.1	3.8	1,030
Lignin, Schwalbe method	VII	5.5	4.9	6.0	7.5	5.0	4.7	14.2	47.8	3.0	22.7	0.2	1,242

* 1 gm. portions of different preparations used in 100 gm. of soil, 20.5 mgm. of nitrogen added to each soil portion, in the form of ammonium sulfate.

TABLE 3

*The decomposition of different fractions of alfalfa meal by the mixed soil flora, in untreated soil**

Preparation	Fraction number	Carbon evolved as CO_2 in: 2 days	4 days	7 days	11 days	16 days	19 days	32 days	Total CO_2	Control soil subtracted	Residual available nitrogen NH_3 - N + NO_3 - N in 100 gm. of soil	Humus, α - fraction in 100 gm. of soil
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Control soil		5.0	....	7.6	4.8	4.2	4.8	15.2	41.6	.....	24.05	612
Untreated alfalfa meal	I	28.8	35.6	22.0	17.9	21.0	19.9	22.4	167.6	126.0	26.80	725
Ether fraction removed	II	28.4	41.8	21.7	16.3	18.4	15.1	27.1	168.8	127.2	28.30	686
Ether and alcohol fractions removed	III	29.6	31.1	19.2	17.9	20.7	13.7	23.8	156.0	114.4	23.80	680
Ether, alcohol, and water-soluble fractions removed	IV	15.4	20.6	18.9	22.0	20.7	14.8	21.2	133.6	92.0	21.30	709
Ether, alkali, water, and alkali-soluble fractions removed	V	14.1	26.4	19.0	17.5	24.3	16.6	39.1	157.0	115.4	14.50	653
Residual cellulose	VI	7.3	17.0	24.9	20.0	26.9	19.0	33.7	148.8	107.2	16.20	667
Lignin by Schwalbe method	VII	4.8	....	7.0	5.3	4.6	6.4	15.5	43.6	2.0	24.30	1,237

* 1 gm. portions of different preparations used in 100 gm. of soil, 20.5 mgm. of nitrogen added to each soil portion, in the form of ammonium sulfate.

slowly and ingredients that are left undecomposed, at least within the short period of time used in the experiment. The results tend also to throw light upon the influence of some constituents (fats, lignins) upon the decomposition of others.

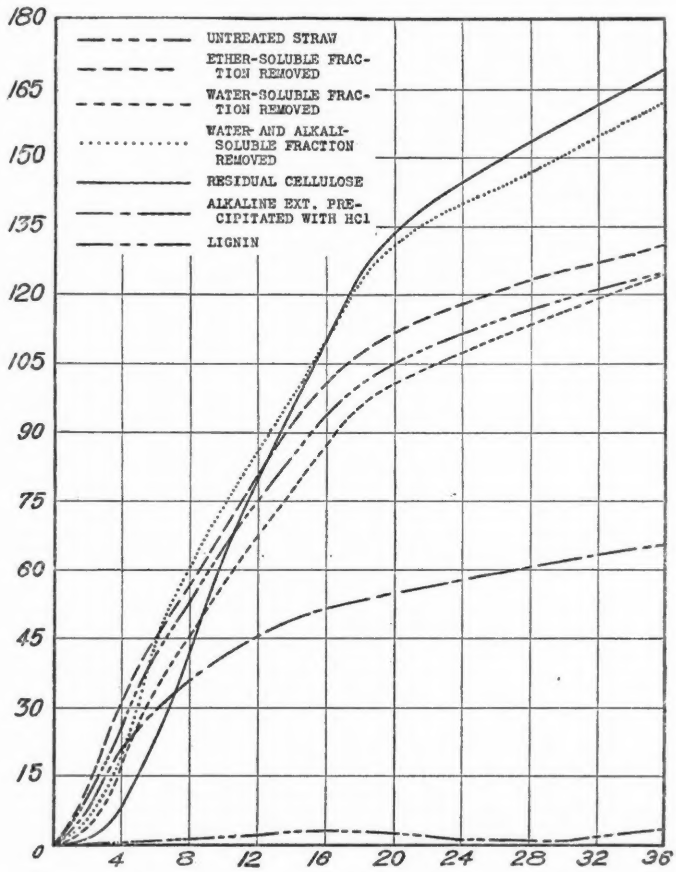

Fig. 1. Course of Decomposition of Cereal Straw and its Various Fractions

The removal of the fats and waxes from the straw hastened somewhat its decomposition, especially at the early stages. The substances removed by ether are not readily acted upon, and they seem to prevent even to a very limited extent the decomposition of the natural organic matter. The same is true, perhaps only to a very limited extent, of alfalfa meal. The treatment of straw with ether and then with 95 per cent alcohol resulted in the

removal of about 4 per cent dry matter. The treated material decomposed at first a trifle more slowly, then only a trifle more quickly than the untreated straw. The favorable effect of the preliminary ether treatment seemed to have been balanced by the unfavorable effect of the alcohol treatment, the latter resulting in the removal of some sugars and amino compounds—sub-

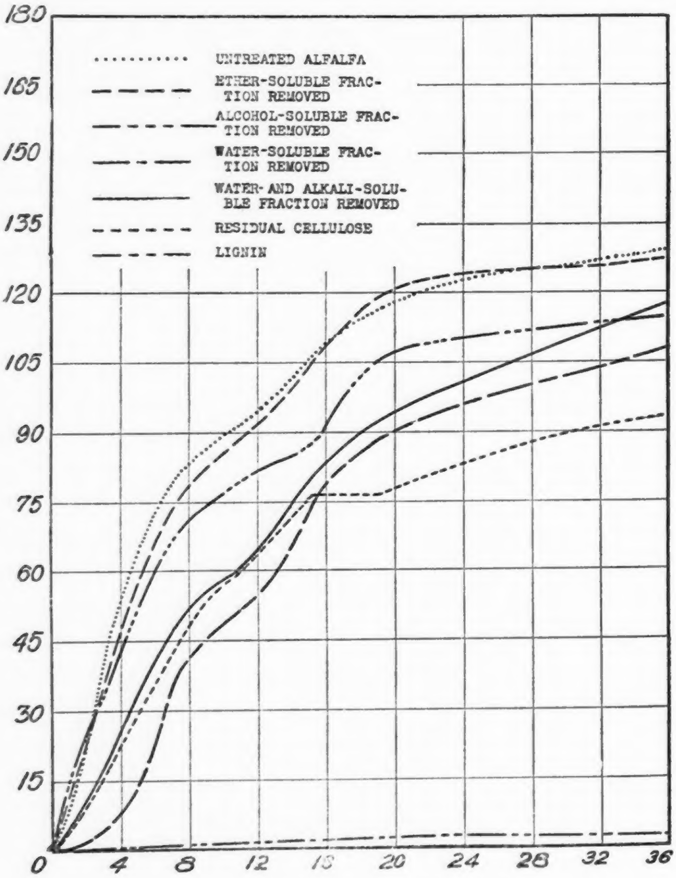

FIG. 2. COURSE OF DECOMPOSITION OF ALFALFA MEAL AND ITS VARIOUS FRACTIONS

stances which are the first to be acted upon when natural organic matter is added to the soil. This is especially marked in the case of the alfalfa meal, which was treated with ether and with alcohol. The preliminary ether treatment did not seem to have a markedly favorable effect; the alcohol, however, removed a considerable amount of reducing sugars and amino acids, namely

the ingredients most readily acted upon. This resulted in a retardation in the decomposition of the organic matter and in a reduction of about 10 per cent in the total amount of CO_2 produced in 35 days from 1 gm. of the preparation.

Since straw contains a comparatively small amount of water-soluble substances (7.46 per cent), one would expect that the removal of these would affect the decomposition of the straw only to a limited extent. The alfalfa meal, however, was found to contain a considerable amount (15.41 per cent) of water-soluble substances and one would expect that their removal would materially influence the rate of decomposition of this organic substance. This was actually found to be the case, especially in the early periods of incubation, since the water-soluble constituents of the natural organic matter are the first to be attacked and decomposed. The presence of a considerable quantity of water-soluble constituents (sugar, starches, amino acids, soluble proteins) in alfalfa meal accounts for the very rapid rate with which this and other legumes are decomposed during the early periods of incubation in the soil, as compared with straw and with cellulose (2, 5).

The removal of 15.41 per cent of water-soluble constituents from the alfalfa reduced considerably its rate of decomposition, as indicated by the evolution of carbon dioxide. The untreated alfalfa gave off 29 and 36 mgm. in 2 and 4 days; the removal of the water-soluble fractions reduced the amount of CO_2 given off (as carbon) to 15.4 and 20.6 mgm. respectively, a rate of decomposition just a trifle lower than untreated straw. In other words, the removal of the water-soluble constituents changes an organic substance with a rapid rate of decomposition, into an organic substance with a slow rate of decomposition. The reason for the more rapid decomposition of alfalfa meal and of other green manures than of straw and of similar organic residues is thus to be looked for in the presence of an abundance of water-soluble constituents in the former.

Alkalies remove from natural organic materials a considerable part of the lignins and a part of the pentosans (and other hemicelluloses). The treatment with hot 5 per cent sodium hydroxide under pressure, of straw and alfalfa from which the ether-, alcohol-, and water-soluble fractions have been removed, did not increase the actual concentration of the materials which decompose rapidly. This was indicated by the fact that the rate of decomposition of the substances left after the alkali treatment, was not at first any greater than the decomposition of those from which the alkali-soluble substances had not been removed. The treatment, however, had a definite influence upon the decomposition of the residual substances, as seen by the fact that the rate of decomposition rapidly increases even within the first few days, soon greatly exceeding the decomposition of the material which has not been treated with hot alkali solution. This is especially true in the case of the straw, where the removal of the alkali-soluble materials increased the total amount of decomposition, in the experiment under consideration, by 33 per

cent. This explains the common practice (3, 1) of boiling straw with alkalies to increase its digestibility. The removal of all or even of only a part of the lignins favors considerably the digestibility of the celluloses by microörganisms. The results of the investigations of the decomposition of straw by pure cultures of microörganisms, reported previously, and by the mixed soil flora are thus found to give comparable results, as far as the influence of the lignins upon the decomposition of celluloses and hemicelluloses in the soil is concerned.

When the organic materials from which the alkali-soluble substances have been removed, are treated further with dilute acids, the decomposition of the residual celluloses is not affected to any considerable extent, for this treatment removes the hemicelluloses (including the pentosans) which have not been removed by the alkali treatment. Both hemicelluloses and celluloses decompose in the soil at about an equal rate and are controlled alike by conditions.

The decomposition of the lignin preparations is especially interesting, since very little is known concerning the decomposition of lignins in the soil. It has been shown previously that lignin prepared directly from organic materials cannot be decomposed by pure cultures of cellulose-decomposing fungi and bacteria. The preparation obtained by precipitating the alkali extract of the soil with hydrochloric acid is not pure lignin but is rich in hemicelluloses, the presence of which accounts for the decomposition of that material in the soil. In these experiments, the extract obtained by treating straw, from which the ether-, alcohol-, and water-soluble constituents have been removed, with 5 per cent hot NaOH under pressure, then precipitating the extract with hydrochloric acid, was found to consist of almost 50 per cent hemicelluloses and about 50 per cent lignin. When this preparation, washed and dried, was added to the soil, it was found to decompose readily at first, then more slowly; the actual amount decomposed was about one-half that of the untreated straw, as shown by the evolution of CO_2. The fact that also about one-half as much nitrogen was used as in the case of the whole straw, shows that there is a definite relation between the CO_2 evolved and the nitrogen assimilated in the whole straw and in its various constituents and also that only about one-half of this preparation was undergoing decomposition. The fact that the other half did not undergo decomposition but went to increase the amount of soil organic matter that does not decompose readily, is borne out by the figures in the column of soil "humus."

The addition to the soil of 1 gm. of lignin, prepared according to the method of Schwalbe, did not increase the rate of evolution of CO_2 from the soil itself. Both lignin from straw and lignin from alfalfa meal gave an increase of only 2 or 3 mgm. of carbon as CO_2 for periods of 35 and 32 days; this quantity of CO_2 lies within the experimental error of the determinations. In other words lignin obtained from straw, and lignin obtained from alfalfa meal practically did not decompose at all in the soil within a period of 35 days. This bears out

the results of various investigators including those of the senior author reported previously that *lignin forms one of the few constituents of the natural organic matter which does not decompose in the soil but accumulates there, thus contributing to the "humus" of the soil.* This is also demonstrated by the actual analyses of the "humus" in the soil.

The amount of available nitrogen assimilated by the organisms in the process of decomposition of natural organic materials is a very good index of the amount of decomposition that has taken place. A definite parallelism between the amount of nitrogen assimilated by the organisms in the soil and the quantity of cellulose decomposed, reported previously, is also well illustrated in table 2, where the amount of nitrogen used up by the microörganism decomposing the straw or its various constituents is exactly parallel to the amount of CO_2 produced, the ratio being in practically all instances 16 or 18 to 1; in other words, 16 to 18 mgm. of carbon is liberated as CO_2 by a mixed soil from straw or its various constituents for every unit of nitrogen assimilated by the microörganisms and changed from an inorganic (as ammonium sulfate) into an organic form. When these results are compared with those previously reported, it is found that the ratio of CO_2 evolution to nitrogen assimilation is 7.0 to 8.0 for pure cultures of fungi, 12.5 for pure cultures of bacteria, and 16 to 18 for the mixed soil flora. The smaller amount of nitrogen used by the mixed soil flora is due largely to the secondary decomposition processes, whereby the protoplasm synthesized by one group of organisms, in the process of decomposition of the carbonaceous materials, is again decomposed by other members of the soil flora, with the result that the nitrogen is again liberated as ammonia and can enter again into circulation. The ratio between the carbon (or energy) decomposed or liberated as CO_2 and the nitrogen assimilated, or protoplasm synthesized, is very definite for the different straw preparations, but varies with the nature of the organisms carrying out the decomposition. This indicates definitely that the nature of decomposition of organic matter, the amount of CO_2 liberated, the amount of nitrogen required for its decomposition, and, probably to a large extent, the "humus" formed, depend largely upon the organisms carrying out the decomposition and upon the environmental conditions favoring the development of the particular organisms rather than upon the nature of organic matter added. Of course the rapidity of decomposition of the organic matter and the nitrogen liberated as ammonia depend upon the composition of the organic matter, which is modified by the nature of the plant and its stage of maturity.

When the data obtained from the decomposition of the various constituents of alfalfa meal are considered in this light, it is found that the decomposition of the whole alfalfa plant does not require any additional nitrogen, but that some is even liberated as ammonia and nitrate (2.75 mgm. nitrogen from 1 gm. of alfalfa meal in 35 days). The removal of the ether- and alcohol-soluble fractions reduced the amount of decomposition by 10 per cent, as pointed out

above, and reduced the nitrogen balance from a gain of 2.75 mgm. of nitrogen, in the form of ammonia and nitrate, to a loss of 0.25 mgm. The removal of the water-soluble constituents and especially of the alkali-soluble constituents, which included most of the proteins of the alfalfa meal, resulted in a preparation similar to straw not only in the decomposition rate but also in the amount of nitrogen required for this purpose. When the "cellulose" preparations of straw and alfalfa meal are compared, there is found in both cases a similar ratio between the carbon of the CO_2 liberated and the nitrogen changed from an inorganic into an organic form, namely 14.46 $\left(\frac{167.8}{11.6}\right)$, in the case of the straw preparation, and 13.66 $\left(\frac{107.2}{7.85}\right)$, in the case of the alfalfa preparation. Differences in the rate of decomposition of various organic materials, as green manures at different stages of growth and residues of different plant and animal origin, can thus be readily explained by the qualitative and quantitative differences in the amount of the various constituents of the different plants, their solubility, etc.

The results of the "humus" determinations are very illuminating. Only the results of the α fraction, or that part of "humus" which is soluble in alkalies (NaOH) and precipitated by acids (HCl) and which is equivalent to the so-called "humic acid," are reported here, since the same soil was used in all cases and the β fractions did not vary greatly. The straw was found to contain 15.5 per cent of lignin by the Schwalbe method and about 18 per cent by the Wilstätter method. By treating the straw with 5 per cent NaOH in the autoclave, only about two-thirds of the lignin is extracted. If the actual lignin content of the straw is assumed to be 18 per cent and that, by treatment with alkali and precipitation with HCl, only about two-thirds of this lignin can be obtained, the latter is quantitatively recovered in the "humus." The addition of 1 gm. of untreated straw to 100 gm. of soil resulted in an increase of 120 mgm. in the fraction of the "humus." When the straw was treated with ether, alcohol, and water, the increase in the humus was 180, 154, and 130 mgm. respectively. When the straw fraction, previously treated with alkali which resulted in the removal of most of the lignin, was added to the soil the increase in the "humus" content in the soil was only 60 mgm. When the preparation was also treated with dilute acids, there was no increase in the "humus" content. In other words, the decomposition of 1 gm. of straw preparation (celluloses and hemicelluloses) from which the lignin fraction had been removed led to an increase of only inappreciable amounts of "humus" in the soil, tending further to confirm the theory that lignin is the ingredient of straw and other natural organic materials which contributes to the soil "humus."

The greatest amount of humus was obtained from the soil receiving the lignin preparations—689 mgm. in the case of the lignin by the Schwalbe method and 447 mgm. in the case of lignin obtained by extraction with NaOH

and precipitation with HCl. When lignin obtained by the Schwalbe method is treated with NaOH and the extract precipitated with HCl, only 70 to 75 per cent of the lignin is recovered. In other words the same amount of lignin prepared after Schwalbe can be recovered from 1 gm. of preparation by direct treatment with NaOH and precipitation with HCl as was obtained from 1 gm. of the preparation which had been added to 100 gm. of soil and incubated under optimum conditions for 35 days. Thus the *evolution of CO_2 shows that lignin does not decompose readily in the soil; the determination of the "humus" content in the soil shows that this lignin is recovered quantitatively in the soil "humus."* In other words, lignin when introduced into the soil is "humus" and both terms are synonymous, to a certain extent.

The "humus" determinations of the soil to which the different fractions of the alfalfa meal were added gave very similar results, although varying somewhat in quantity. The actual increase in the amount of "humus" recovered from the addition of 1 gm. of lignin from alfalfa meal was 625 mgm.—very similar to the figure obtained for the lignin of the straw and by extracting lignin directly with alkali and reprecipitating the extract with acid. This is due to the fact that lignin dissolves very incompletely in alkalies under ordinary pressure. It is important to keep in mind, in this connection, that the actual "humus" extracted from the soil by treatment with alkalies, is never larger than 60 to 80 per cent of the total soil organic matter, the insoluble part frequently being referred to as "humin."

It is interesting in this connection that van Suchtelen (6) found that when natural organic substances are added to the soil, the monosaccharides and pentosans are decomposed first, followed by the celluloses, pectins, starches and proteins; a strongly resistant carbonaceous residue is left which is decomposed only very slowly. The results presented in this and in the previous paper tend to confirm this as well as the ideas of other workers (7) that lignins are very resistant to decomposition by microörganisms and that they are the mother substances of soil "humus."

SUMMARY

1. The removal of ether-soluble fractions from barley straw was found to hasten somewhat the decomposition of the straw, but the treatment was without influence upon the decomposition of alfalfa meal.

2. The removal of alcohol- and water-soluble substances was of little influence upon the decomposition of the straw materials but greatly reduced the rapidity of decomposition of alfalfa meal.

3. The removal of the lignins from straw and alfalfa meal hastened the rapidity and increased the amount of decomposition of the residual materials.

4. Lignins are not decomposed in the soil, at least within the experimental period of 32 to 35 days; if they are decomposed at all, the amount of decomposition is only insignificant in comparison with the decomposition of the other constituents of natural organic matter.

5. The lignin introduced into the soil was recovered practically quantitatively at the end of the incubation period as soil "humus," allowing for the imperfection of the method of extraction of the lignin and "humus" from the soil.

REFERENCES

(1) ARCHIBALD, J. G. 1924 The effect of sodium hydroxid on the composition, digestibility, and feeding value of grain hulls and other fibrous material. *Jour. Agr. Res.* 27: 245–265.

(2) DVORAK, J. 1912 Studien über die Stickstoffanhäufung im Boden durch Mikroorganismen. *Ztschr. Landw. Veruchsw. Osterr.* 15: 1077–1121.

(3) MAGNUS, H. 1919 Theorie und Praxis der Strohaufschliessung. Berlin.

(4) SCHWALBE, H. 1925 Eine neue Methode zur Bestimmung des Lignins. *Papier Fabrik.* 23: 174–177.

(5) STARKEY, R. L. 1924 Some observations on the decomposition of organic matter in soils. *Soil Sci.* 17: 293–314.

(6) VAN SUCHTELEN, F. H. H. 1910 Über die Messung der Lebenstätigkeit der aërobiotischen Bakterien im Boden durch die Kohlensäureproduction. *Centbl. Bakt.* (II) 28: 45–89.

(7) WAKSMAN, S. A. 1926 The origin and nature of the soil organic matter or soil "humus." I. Introductory and historical. *Soil Sci.* 22: 123–162.